Yeasts Associated With The Worker Caste of The Leaf-Cutting Ant Atta Cephalotes Under Experimental Conditions In Colombia

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Abstract

Yeasts isolated from the worker caste of the Colombian leaf-cutting ant, *Atta cephalotes* (Hymenoptera: Myrmicinae) were cultured and identified by molecular methods. Abundant, persistent and omnipresent species were classified as “prevalent”. Experimental data were compared with information gathered from published reports on the yeast species composition in other leaf-cutting ant species. Diversity analysis was conducted using diversity values (q0, q1, and q2) to compare richness and abundance of yeasts present in different leaf-cutting ant species Clustering analysis was carried out to assess similarity of yeast community according to ant species. The yeast species composition was highly variable among the ant species. *Atta laevigata* and *A. capiguara* showed the highest degree of similarity and differed from the group composed by *A. cephalotes, A. sexdens, A. sexdens rubropilosa*, and *A. texana.* The isolation of dominant yeasts in different ant castes within the different compartments of a colony strongly suggests that the identified microorganisms are not transient but are native to the soil surrounding ant colonies and the substrates used by the ants to grow their fungal cultivars. It is apparent that the ant-fungus mutualism does not operate in an environment devoid of other microbes, but rather that the association must be seen within the context of a background of other microorganisms, particularly the dominant yeasts.

Introduction

Although the link between ascomycete yeasts and ant communities has long been known, our knowledge regarding the nature, species partners, mechanisms and trade-offs of these associations is limited; and, not surprisingly, most information about these associations remains elusive. The association between yeasts and ants has received less intensive study compared with that in subsocial insects like ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) and social insects such as bees (Golonka 2002; Brysch-Herzberg and Lachance 2004), wasps and termites (Suh et al. 2005; Stefanini 2018). In any event, these associations appear to be evolved or mediated for a set of diffuse mutualisms (Madden et al. 2018). In the subtribe Attina (Hymenoptera: Formicidae), which includes the leaf-cutting ants (LCA) in the genera *Acromyrmex*, *Atta*, and *Amoimyrmex*, the picture is even more complicated, given that they represent a great example of mutualistic relationship with *Leucoagaricus gongylophorus* (Möller) Singer 1986 and *Leucoagaricus* spp., basidiomycete fungi (Pagnocca et al. 2012; Mueller et al. 2017).

LCAs are regarded as the most highly advanced insects in terms of social organization (Erthal et al. 2007) and cultivate the fungus that represents their sole food source. The fungus needs to be constantly supplied with freshly cut plant material to grow (Mueller and Rabeling 2008). In return, ants disperse and provide the fungus with a protected environment. The latter task is performed by the secretion of their metapleural glands and by the association of beneficial bacteria such as actinobacteria and *Burkholderia* sp. (Bot et al. 2002; Santos et al. 2004; Fernández-Marín et al. 2009; Li et al. 2018) that help ants to inhibit and remove potential contaminants. All of these mechanisms have been examined experimentally. Currie et al. (2003), Poulsen et al. (2003) and Santos et al. (2004) revealed that this symbiotic interaction is more complex than a simple bipartite association involving yeasts as fundamental partners. The association between a black yeast (*Phialophora*-like), classified by Little and Currie (2007) as the fifth symbiont, and *Apterostigma* ants (subtribe Attina) was the first to be discovered. Later, three new black fungal species isolated from LCAs were unraveled: *Phialophora attinorum*, *P. capiguarae* (Attili-Angelis et al. 2014) and *Ochroconis globalis* (Samerpitak et al. 2015). More recently, Duarte et al. (2014; 2017) isolated several species of black fungi in the Teratosphaeriaceae family from LCAs.

These associations are not surprising because yeasts are among the most abundant and omnipresent microorganisms in nature, specially associated with insects (Fernández 2003; Poulsen et al. 2003; Rodrigues et al. 2009; Rosa and Gábor 2006; Rosa et al. 2010). A question that has been addressed is the role of yeasts in the complex symbiotic interactions that LCAs establish in diverse environmental conditions. It has been thought that some yeast species may provide essential nutrients, detoxify plant compounds that may be toxic to the ants (Mendes et al. 2012), and they are even suspected to act as competitors against actinobacteria that are located on the ants’ integuments (Little and Currie 2007; 2008). However, many of these associations are poorly understood or there is a large gap in our current understanding of LCAs yeast diversity, which is far from being bridged. Hence, it is expected that yeasts are part of the complex interactions in LCA colonies (Duarte et al. 2017).

Most of the attine ant-associated yeast studies have been conducted on ants of the genus *Atta*. In a pioneering study, Carreiro et al. (1997) found 34 species of yeasts in seven genera: *Candida, Cryptococcus, Rhodotorula, Sporobolomyces, Tremella, Trichosporon* and *Pichia* in *Atta sexdens rubropilosa*. Pagnocca et al. (2008), investigating the yeasts associated with the exoskeleton, pellet and infraoral pocket of queens of *A. capiguara* and *A. laevigata* found *Auerobasidium pullulans, Rhodotorula glutinis, Cryptococcus laurentii* and *Candida parapsilosis*. Likewise, Middelhoven et al. (2003) and Carreiro et al. (2004) found *Cutaneotrichosporon haglerorum* (= *Cryptococcus haglerorum*) and *Blastobotrys attinorum* (= *Sympodiomyces attinorum*), respectively, associated with *Atta sexdens*, while Little & Currie (2007; 2008) found the black yeast *Phialophora* sp. on *Apterostigma* sp. In *A. texana* by in vitro assays, Rodrigues et al. (2009) showed that yeasts may be important as disease-suppressing agents in the ant gardens. Some research has also shown the association of yeasts in colonies of ants in the genus *Acromyrmex*. Masiulionis and Pagnocca (2016) found *Wickerhamomyces spegazzinii*, an ascomycete yeast associated to the fungus garden of *Acromyrmex lundii*, and Melo et al. (2014) registered the presence of *Starmerella aceti*, associated with *Acromyrmex balzani*. Della Lucia et al. (2014) reviewed yeast species associated with several Brazilian LCA species (i.e. *A. capiguara*, *A. laevigata* and *A. sexdens rubropilosa*).

In Latin America, *Atta cephalotes* is the most extended LCA species and probably the most economically important (Della Lucia et al. 2014). In Colombia, it is dominant, causing large economic loses by attacking agricultural crops and destabilizing building and construction projects

(Montoya-Lerma et al. 2012). However, despite its importance, studies on the yeast mycobiota of this species are scarce and/or have not been published. In particular, yeasts have either been missed or neglected by current studies.

In a pioneering work, Craven et al. (1970) detected the presence of yeasts in the fungus gardens of *A. cephalotes* and *Ac. octospinosus*, using electron microscopy and microbiological techniques. These yeasts were likely native to Panama. However, identification was not carried out. Fisher et al. (1996) reported 18 taxa and several sterile mycelia and yeasts isolated from the fungus gardens of *A. cephalotes*, collected in Trinidad and Tobago. Although no attempts were made to distinguish among different taxa, yeasts were scored between 20 and 40% of the total isolates found in the leaf substrate. In attempts to find the presence of *Escovopsis* in colonies of this LCA species, as well as several bacteria, Giraldo (2009) found and isolated both filamentous fungi and yeast species on the worker caste. The results provided valuable information as to the isolated, cultured and identified yeast species from *A. cephalotes*. Further, this author determined the detailed distribution of these microorganisms on different worker subcastes within the colony and hypothesized that the presence of particular microorganisms should reflect an adaptive biological strategy linked to the division of labour and asepsis of the colony. The latter might have important biotechnological impacts due to the capacity of some yeast (e.g. the killer phenotype) to secret toxins that control other microorganisms as reported by Robledo-Leal et al. (2016).

Here we present the results of a study directed to establish the yeasts species associated to *A. cephalotes*. Data is compared with current information on the yeast composition reported for other LCA species.

Methods

Ant colonies

Four *A. cephalotes* colonies were obtained from two sites in Cali, Colombia: the Universidad del Valle campus (3º 22´23.07´N 76º31´50.69´´W; 1,000 m above sea level) and the rural area of Pichindé (Entre Quebradas: 3º24´78´´N 76º35´30.69´´W and Corral de Piedra: 3º24´51.84´´N 76º35´36.39 ´´9). Both sites are located at 1,350 m.a.s.l with an average relative humidity of 80% and an average temperature of 23 ºC. All colonies were maintained for two years in the Entomology Laboratory at the Universidad del Valle, Cali-Colombia, at 24°C and 95% relative humidity, using standard techniques (Valderrama et al. 2006). In a completely random design with two food supply sources, two ant colonies were fed on fresh mango (*Mangifera indica*) leaves and two were fed on commercial oat flakes.

Yeast counts and isolation

At 30-day intervals, small food fragments and three worker ants per labour category (cutters, carriers, gardeners, nurses, soldiers and dumpers) were sampled. During sampling, each ant was aseptically cleaned and submerged in 5% peptone liquid medium, which was then split into three different test tubes. After incubation at 37°C, for 12 hours and a process of distress, serial dilutions were made on 5% peptone medium for 6 hours. After that, decimal dilutions in sterile physiological solution (NaCl 9.0 g L^{-1}) were made and 0.1 mL of each dilution was spread on two different media: Wallerstein Laboratory nutrient agar (WLN; Oxoid, Milan, Italy) and yeast peptone dextrose agar (YPD; 1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose and 2% w/v agar). All media were supplemented with chloramphenicol (150 ppm). Plates were incubated at 25°C for 3-5 days. After counting, all the persistent colonies were isolated from WLN and YPD plates. The isolates were purified by repetitive streaking on YPD and then stored at -20°C in YPD broth supplemented with glycerol (25% final concentration). Viable yeasts were measured by counting the number of colony-forming units (CFU). A total of 5184 ants were collected from different worker castes.

Yeast identification

Yeast identification was performed combining internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) and 26S rRNA barcoding analysis. Yeast cells were grown aerobically in YPD at 28°C. DNA was isolated according to Querol et al. (1992). The ITS was amplified in a MyCycler (Bio-Rad Laboratories, Milan, Italy) using the primers ITS1 and ITS4 as described previously (Esteve-Zarvoso et al. 1999; Tofalo et al. 2012). The amplified DNA was digested with the restriction endonucleases *Hinf* I, *Cfo* I and *Hae* III (Roche Diagnostics, Mannheim, Germany) according to the supplier's instructions. The PCR products and their corresponding restriction fragments were separated in 1.5% and 2% agarose gels, respectively, in a 1× TAE (40 mM tris-acetate, 1 mM EDTA, pH 8.2) buffer. After electrophoresis, gels were stained with ethidium bromide and documented by the Gel Doc 2000 (Bio-Rad).

Amplification of the D1/D2 domains of the 26S rRNA gene was carried out for representative yeast isolates from the obtained ITS-RFLP profiles. Amplicons were obtained using primers NL1 and NL4 as described by Kurtzman & Robnett (1998). PCR products were gel purified with GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences AB, Uppsala, Sweden) according to the manufacturer's instructions and delivered to BMR genomics (Padua, Italy) for sequencing. The sequences were compared with those deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/) using Blast (Altschul et al. 1997).

Phylogenetic analysis

The 26S rRNA gene sequences were aligned using Muscle (Edgar 2004) and a phylogenetic tree was constructed by using the Neighbor-Joining method. The bootstrap analysis was conducted on 1000 replicates. The sequence analyses were performed with Mega 7 (Kumar et al. 2016).

LCA yeast composition and comparison

We used the coverage estimator recommended by Chao & Jost (2012) to estimate the accuracy of inventories:

$$\widehat{C}_n = 1 - \frac{f_1}{n}\left(\frac{(n-1)\,f_1}{(n-1)\,f_1 + 2f_2}\right)$$

where f_1 and f_2 are the numbers of species with one individual (isolate) and with two individuals in the sample, respectively, and n is the number of individuals. We determined species diversity metrics using Hill numbers (i.e., numbers equivalent, *sensu* Jost 2006). We used the Hill number of order 0 (^0D, species richness), 1 (^1D, exponential of Shannon's entropy), and 2 (^2D, inverse Simpson concentration). ^{0}D is not sensitive to species abundances and thus gives disproportionate weight to rare species (Jost 2006). ^1D weighs each species according to its abundance in the community; hence it can be interpreted as the number of 'common' or 'typical' species in the community (Jost 2006). Finally, ^2D can be interpreted as the number of 'very abundant' or 'dominant' species in the community (details in Jost 2006).

Results

Yeast isolation

During our microbiological investigation of ant workers and food substrates several different microbes grew on culture media, including bacteria, filamentous fungi and yeasts. A total of 144 microbial morphotypes was found in *A. cephalotes* colonies, but only 52 species of dominant and persistent microorganisms were observed, including 27 bacteria, 8 filamentous fungi and 17 yeasts. Bacteria and filamentous fungi were taking into account only for comparison purposes but they were not accounted in our diversity analysis.

We obtained a total of 30 yeast isolates which were grouped by ITS-RFLP analysis (Table 1). The strains showed different PCR product sizes, ranging from 400 to 600 bp (Table 1). Seven different profiles, named from I to VII were obtained, which were compared to the data set previously described (Esteve-Zarzoso et al. 1999; Villa-Carvajal et al. 2006; de Llanos Frutos et al. 2004). The isolates with profile I showed a pattern similar to those reported in literature for *Candida intermedia* (de Llanos Frutos et al. 2004). The other profiles did not allow obtaining an unambiguous identification on the basis of already published RFLP profiles (Esteve-Zarzoso et al. 1999; Villa-Carvajal et al. 2006; de Llanos Frutos et al. 2004). To achieve a certain identification of these yeasts, the D1/D2 domains of the 26S rRNA gene was sequenced and compared with those available in GenBank. All the sequences obtained displayed similarity values ranging from 99 to 100% to reference sequences (Table 1). According to barcoding analysis using the D1/D2 domain, isolates with atypical restriction pattern were assigned to *Hyphopichia burtonii* (For6, For16, For30, For31, For18) and *H. homilentoma* (For11) [profile II]; *Trichosporon coremiiforme* (For24, For25, For29) [profile III]; *Candida tropicalis* (For17) [Profile IV]; *Debaryomyces hansenii* (For2, For19, For28) and *D. nepalensis* (For33) [Profile V]; *Candida* spp. (For22, For23, For21, For20) [profile VI], *Candida carpophila* (For3), *Meyerozyma guillermondii* (For4, For9, For32) and *Pichia* spp (For15) [profile VII].

Table 1
Sizes of the 5.8S ITS rRNA gene amplicons and the respective restriction fragments profiles of yeasts isolated from *Atta cephalotes*. Strains were sequenced (in bold) for LSU barcoding identification.

Strain ID	PCR product[a] (bp)	Restriction fragments (bp)			ITS identification	ITS profile	26S identification[b]	Similarity (%)	Accession number
		Cfo I	Hae III	Hinf I					
For1-For5-For7-For8-For12-For13-For14-For27	400	209+179	400	206+190	*Candida intermedia*	*I*			
For6-For16-For30-For31-**For18**	450	450	450	219+179	*Hypopichia burtoni*	*II*	*Hyphopichia burtonii*	99	KC192660
For11	450	450	450	219+179	*Hypopichia burtoni*	*II*	*Hyphopichia homilentoma*	99	KY106501
For24-**For25**-For29	500	265	500	219+179	*Trichosporon coremiiforme*	*III*	*Trichosporon coremiiforme*	99	KY109941
For17	500	289+239	450+70	219+179	*Candida tropicalis*	*IV*	*Candida tropicalis*	99	MF148862
For2-For19-**For28**	600	300+300	396+112	317+317	*Debaryomyces hansenii*	*V*	*Debaryomyces hansenii*	100	LC219505
For33	600	300+300	396+112	317+317	*Debaryomyces hansenii*	*V*	*Debaryomyces nepalensis*	99	KY107577
For 22-**For23-For21-For20**	600	263+160+124	394+112	317	.	*VI*	*Candida* sp.	99	HQ014450
For3	600	275+239	367	317+286	-	*VII*	*Candida carpophila*	99	KY106385
For4-For9-For32	600	275+239	367	317+286	*Pichia guillermondii* /*Pichia caribbica*	*VII*	*Meyerozyma guillermondii*	99	JQ686904
For15	600	275+239	367	317+286	*Pichia guillermondii* /*Pichia caribbica*	*VII*	*Pichia* sp.	100	JX125040

[a] 5.8S-ITS amplified product size in bp

[b] D1/D2 region sequencing.

Multiple sequence alignment and phylogenetic analysis were carried out to study the evolutionary relationship among the species and of the reference strains showing the distance between taxa. The phylogenetic tree is shown in Figure 1. All the strains clustered together with the corresponding species, confirming the identification.

Abundance and diversity of yeasts

With respect to the persistent yeast in the colony, the greatest diversity (q0, q1 y q2) was found in the caste of garden ants (Figure 2). Waste-transporting ants (WT) had the lowest richness (q0). Yeasts were nearly absent from workers found in the refuse material (Figure 2). Clustering analysis comparing the data found in the present study with data published in previous report suggests that the ants *A. laevigata* and *A. capiguara* have a greater similarity in terms of diversity of yeasts (Figure 3). On the other hand, *A. cephalotes* is closer to *A. sexdens* and *A. sexdens rubropilosa*. The most distant species of the group is *A. texana* (Figure 3).

Discussion

Several studies have found yeast species associated with fungus gardens of leaf-cutting ant colonies (Carreiro et al. 1997; 2004; Little and Currie 2007; 2008; Masiulionis and Pagnocca 2016; Melo et al. 2014; Middelhoven et al. 2003; Rodrigues et al. 2009). However, it is interesting that yeast composition is variable among *Atta* species. In a pioneering study, Carreiro et al. (1997) found 34 yeast species associated with *Atta sexdens rubropilosa*, grouped in seven genera: *Candida, Cryptococcus, Rhodotorula, Sporobolomyces, Tremella, Trichosporon* and *Pichia*. Recently, Arcuri et al. (2014) updated this information by finding a low number of orders (six) and species (25) but increasing the number of genera (10). Our results

coincide with those of these authors, revealing that there is an unequal distribution of yeasts in the different elements of the colony (e.g. foliage, new fungus, old fungus, workers' bodies, nest floor, and waste material) and that some occur frequently, while others are intermittent.

Also, the results of the present study are also similar to that of Carreiro et al. (1997) in recording the genera *Candida, Trichosporon* and *Pichia* and the species *C. homilentoma* (now *Hyphopichia homilentoma*) as prevalent yeasts. It is important to mention that similar to what was recorded in this study, these authors found the species *C. homilentoma*, *C. robusta*, *C. sake*, *C. valida*, *Candida* sp., *Pichia anomala* (now *Wickerhamomyces anomalus*) and *Trichosporon beigelii*, on the integument of workers. Therefore, both studies reinforce the hypothesis that yeasts are not only found in the fungus gardens but can also be part of the microbiota of workers, most likely fulfilling certain physiological functions.

When investigating the yeasts associated with the integuments, pellet and infrabucal pocket of queens of *A. capiguara* and *A. laevigata*, Pagnocca et al. (2008) found *Aureobasidium pullulans*, *Rhodotorula glutinis*, *Cryptococcus laurentii* (now *Papiliotrema laurentii*) and *Candida parapsilosis*. Similarly, Angelis et al. (1983) and later Carreiro et al. (1997), isolated 13 yeast species from the nests of *A. sexdens rubropilosa* and *A. laevigata* from the genera *Candida*, *Torulopsis*, *Rhodotorula* and *Kloeckera*. Also, Middelhoven et al. (2003) and Carreiro et al. (2004) found *Cutaneotrichosporon haglerorum* and *Blastobotrys attinorum*, respectively, associated with leafcutter ants, while Little and Currie (2007; 2008) found the black yeast *Phialophora* sp. related to *Apterostigma* sp. However, none of these species are coincident with those found in the present investigation. It should be noted that none of aforementioned authors sampled the workers considering caste separation.

Although *Rhodotorula* was found in attine ants by Angelis et al. (1983) and later on by Carreiro et al. (1997) and Pagnocca et al. (2008), this genus was not found in the workers of *A. cephalotes* in our study. On the other hand, *Candida* was present consistently in all the investigations mentioned, and presented a large variation and diversity of species, which allows us to infer a possible persistent association between

In the present study, the fact that genera and species were found exclusively in the garden caste, including *Candida intermedia*, *Candida* spp., *C. fermentati*, *Trichosporon coremiiforme*, *Yamadazyma mexicana*, *H. burtonii* and *D. hansenii* has special relevance. The isolation of large numbers of yeasts from the garden workers justifies the study of these organisms as being potentially associated with different processes in the colony. Also, it is necessary to deepen these investigations to understand whether these microorganisms are directly associated with the bodies of the ants or if they are exclusively found in the fungus garden and then transported by the workers when they come in contact with the mutualistic fungus.

Carreiro (2000) hypothesized that both yeasts and ants may benefit from their relationships. They posit that while yeasts are taking advantage of the nutrients available in the fungus garden, ants might benefit from the enzymes that yeasts secrete into the fungus garden, as they would contribute to the digestion of the plant substrate collected by the ants. These enzymes may also contribute to the degradation of plant tissue by the fungal cultivar by breaking down structural plant polysaccharides such as pectin and hemi-cellulose (Carreiro 2000; Pagnocca et al. 2010; Mendes et al. 2012). The elucidation of the pathways of these processes (and if yeasts in fact participate on those) may render great potential biotechnological applications. However, the identification of species biodiversity, which is a basic preliminary step, has not been completed in our study. Further studies are required to elucidate the role of isolated yeast species in the framework of the complex mutualistic system hitherto described.

At least 17 species of yeasts have ecologically diverse associations with ants (Rosa and Gábor 2006). Many are mutualistic but not completely understood (Suh et al. 2008) while others, called killer yeasts, are capable of secreting protein metabolites of variable molecular weights named killer toxins, which are capable of inhibiting other microorganisms by altering cell walls, membranes, or vulnerable cell nuclei (Buzzini et al. 2007), which in the case of the killer phenotype of *Torulaspora globosa* strain 1S112 in the *Atta* genus is under *in vitro* and *in vivo* evaluations (Robledo-Leal et al. 2016). Among the yeasts identified, there were several species reported as antagonistic or effective as potential killer yeasts for the control of fungi that cause plant deterioration. These yeasts might have great potential for biotechnological applications in diverse areas, especially in agriculture, where better results might be obtained in the biological control of phyto-pathogens (Ahansal et al. 2008; Rosa et al. 2010). For instance, the fungus *Botrytis cinerea*, one of the most harmful pathogens in grape and strawberry crops, was inhibited using a partially purified strain of *Pichia membranifaciens* CYC 1106 (Santos and Marquina 2004). This same fungus was later inhibited using strains of *Pichia anomala* and *Debaryomyces hansenii*, increasing the biological control arsenal for this pathogen (Santos et al. 2004). Strains of *Issatcehnkia orientalis*, *Candida guilliermondii* (now *Meyerozyma guilliermondii*), *P. ohmeri* and *Torulaspora globosa*, among others, have been reported to successfully inhibit important plant pathogenic fungi, such as *Aspergillus cabonarius*, *A. nig*er, *Penicillium expansum* and *Colletotrichum sublineolum* in grape, pear, apple, and sorghum crops, respectively (Bleve et al. 2006; Coelho et al. 2009; Rosa et al. 2010). In Mexico, Hernández-Montiel et al. (2011) reported a significant reduction of *Geotrichum citriaurantii* in limes post-harvest, using epiphytic *D. hansenii* yeasts.

Several authors (Currie et al. 1999; 2003; Poulsen et al. 2003; Santos et al. 2004) previously stated the need to challenge the misconception of an axenic culture (monoculture) of the mutualistic fungus of leaf-cutting ants. They also suggested that symbiotic interactions cannot be viewed as bipartite associations and alleged the presence of a third additional organism. Our results support a well-conceptualised idea that the fungus gardens are home of a diverse and complex microbiota. Furthermore, the fact that we found abundant and dominant yeasts associated with the worker caste highlights the importance of the presence of these neglected but potential symbiotic organisms in the ancestral *Atta cephalotes*-fungal cultivar mutualism. Hence, the mutualistic network within this system could be more complex than previously thought. Confirmation of this issue is challenging, although with the advent of novel technologies more precise and detailed identification of the yeasts is achievable. Knowing

the species identity might provide valuable information that helps to elucidate the role of these microorganisms as poly-associates, as the composition of many of these associations depends on the microbial diversity of the environment their colonies inhabit. Future work on *A. cephalotes* or other leaf-cutting ants should focus on the extent and similarities of microbiota and also should address whether this biota has a role in the division of the labour within their colonies.

Declarations

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Conflict of interests

The authors declare no conflict of interest in this article.

Author Contribution Statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Carolina Giraldo. The first draft of the manuscript was written by Carolina Giraldo and James Montoya-Lerma. All authors commented and contributed to previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

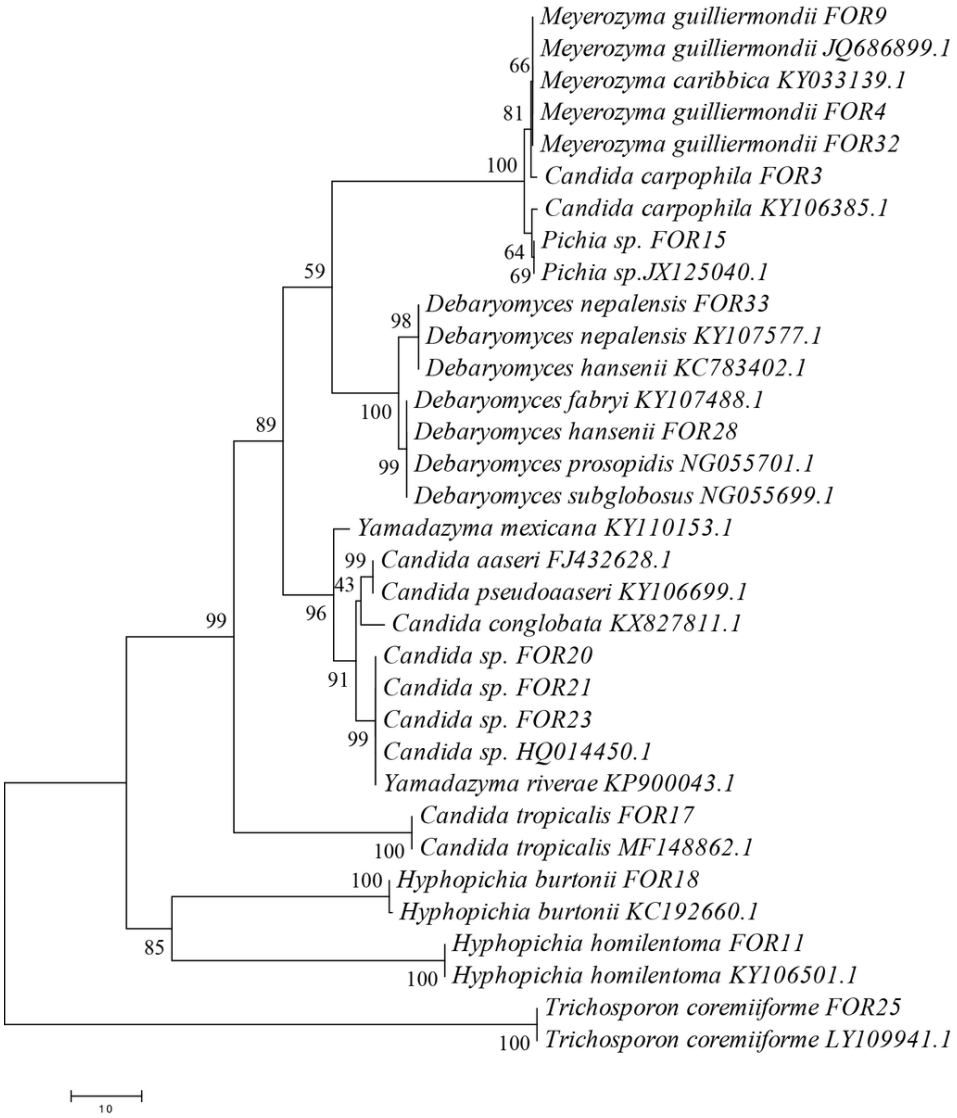

Figure 1

Phylogenetic tree depicting evolutionary relationships among isolates and related reference species as determined by partial 26S rRNA gene sequence analysis. Bootstrap values reported at the nodes in percentages indicate support of the analysis.

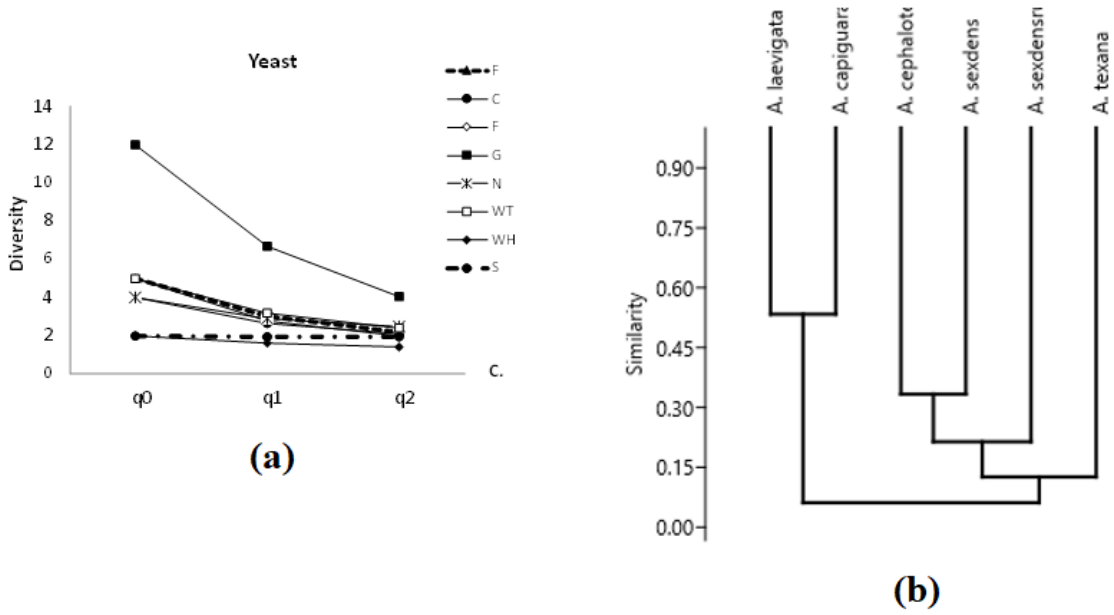

Figure 2

a. Comparison of yeast species diversity by Hill numbers of order 0D, 1D and 2D in different castes. F: Forage ants; C: Cutters; G: gardeners; N: nurses; WT: Waste-transporting; WH: dumpers and S: soldiers b. Clustering analysis of yeast diversity found in different Atta species using Jaccard similarity index.