Phylogenetic community structure of ants in secondary tropical forests in Brazil.

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For my daughter Eleanor Wangeci Nyoike

Erklärung

Ich versichere, daß ich meine Dissertation **„Phylogenetic community structure of ants in secondary tropical forests in Brazil."** selbständig und ohne unerlaubte Hilfe angefertigt habe und mich keiner als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe. Diese Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Rossa Ng'endo Nyoike

Marburg.

To be willing is to be able.

(Source: French)

TABLE OF CONTENTS

Chapter 1

1 General Introduction

1.1 Tropical forests biodiversity and community organization

Tropical forests are one of the most diverse habitats on Earth (Whitmore, 1998), hosting at least two-thirds of the Earth's terrestrial biodiversity (Gardner et al., 2009). However, the future of tropical forest species has been more uncertain since only few areas of the tropics have escaped some form of human impact (Kareiva et al., 2007). The combined influence of persistently high rates of deforestation and forest degradation, over harvesting, invasive species and global environmental change threatens to make tropical forests the epicentre of current and future mass species extinctions (Bradshaw et al., 2009), if current rates of deforestation continue (Pimm et al., 1995; Dirzo & Raven, 2003).

Deforestation of tropical forests is rarely total or permanent. Small patches of original habitat remain and succession leads to secondary forests. Succession involves a gradual replacement of species that differ in traits (which allow for quick colonization or competitive ability) and that differ in the degree they tolerate, facilitate or inhibit certain environmental conditions and other species (Rossi et al., 2009). Therefore, it is an important process in determining how, and how fast, ecological communities return back to their original state, especially in terms of biodiversity composition in recovering ecosystems such as secondary forests. Ecological communities refer to assemblages of species that occur together in time and space, and whose composition and aspect is determined by the properties of the environment and by the relations of the organisms to each other (modified from Begon et al., 2006), while an assemblage refers to a taxonomic subset of a community (Fauth et al., 1996).

With the continued increase in secondary forests throughout the tropics, conservation has a key role to play in safeguarding the future of tropical forests

biodiversity (Gardner et al., 2010; Bihn et al., 2008, 2010). However, a major challenge to conservation is due to the large number of undescribed species in the tropics, especially the invertebrates (Gentry, 1992; Hodkinson & Casson, 2008), with conservation prioritization having largely focused on vertebrates. Therefore, species identification of such large numbers of undescribed taxa forms the first step towards achieving conservation agenda.

Human-induced environmental change in forest ecosystems has also been linked to significant changes in the species composition of communities, with implications for the persistence of ecological communities and ecological processes of even the most remote and pristine areas of tropical forest (Lewis et al., 2009). Community composition is influenced by a range of ecological factors (dispersal ability and habitat selection of a species, interspecific interactions), evolutionary processes and historical events (Morin, 1999). Since differences in ecological characteristics of species lead to differences in their functionality and their role in ecosystem processes (Darwin & Wallace, 1858; Loreau et al., 2001), there have been questions on which factors structure local communities; and how regional species pools contribute to local communities continue to be among the central topics in ecology (Diamond, 1975). Starting from the early studies of Hutchinson (1959) and MacArthur (1958; MacArthur, 1972), research efforts in community ecology have attempted to reveal the mechanisms that allow the coexistence of species in local habitats and ecosystems (Brown 1995).

A range of mechanisms has been suggested in order to understand the underlying reasons for the co-occurrence of closely related, ecologically similar species (e.g., Chesson, 2000; Hubbell, 2001; McPeek, 2000). Furthermore, community studies have also focused on how factors such as spatial or taxonomic scales, null models and metrics among others, may influence the observed community patterns (Webb et al., 2002; Cavender-Bares et al. 2006). Moreover, recent research studies have emphasized the adoption of biodiversity indices that take into account phylogenetic information of species, when assessing dynamics in species composition and community patterns (Webb et al., 2002; Chave et al., 2007). This is expected to allow for better interpretation of e.g. effects of anthropogenic impacts on phylogenetic composition/structure of ecological communities as opposed to the case with classical indices (see Warwick & Clarke, 1995, 1998; Anu & Sabu, 2006).

To establish the absolute effect of succession on communities in secondary tropical forests, we need to know species diversity in order to examine phylogenetic composition and how communities are organized along the succession gradients. In this study, I first assessed the diversity of a hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae) in Rio Cachoeira Nature Reserve found in the tropical secondary forests of Brazil. Next, I examined the phylogenetic composition/ relatedness and structure of ant communities in this genus, and the processes structuring these communities. I also looked at how succession influences phylogenetic composition of species and community patterns. Lastly, I examined the phylogenetic structure of ant genera occurring in the tropical secondary forests and related this to the patterns observed when using species in the genus *Pheidole* alone.

1.2 Biodiversity of a hyperdiverse ant genus in secondary tropical forests

Globally, loss of tropical forest habitats has been identified as a major threat to biodiversity (Jenkins, 1992; Whitmore & Sayer, 1992; Pimm & Raven, 2000). This is likely so because most biodiversity is extraordinarily concentrated in tropical forests and habitat loss is widely anticipated to lead to a mass extinction of species if current rates of deforestation continue (Pimm et al., 1995; Dirzo & Raven, 2003). The biodiversity value of secondary forests in the tropics is an area of much uncertainty because most species of small-bodied taxa, which account for the vast majority of species in these forests, are under-described (Bihn et al., 2008). In addition, secondary forests are increasing in extent and importance as forest habitats throughout the tropics, and with the increased land-use dynamics; they are highly vulnerable to human degradation (Chazdon et al., 2009).This raises concerns over the threats to biodiversity and the desire to accurately describe and monitor as many species as possible before they disappear (Wiens, 2007).

A biodiversity survey using an invertebrate group would be of much relevance in regenerating forests owing to the major roles they play in ecosystems. For instance, ants (Hymenoptera: Formicidae) are a dominant invertebrate group in tropical forests, and their strong interactions with other organisms make them important —keystone species in tropical forests (Bihn et al., 2008). Furthermore, ants structure their environment through their roles as seed dispersers (Beattie, 1985; Levey & Byrne, 1993), predators (Andersen, 1992; Kaspari, 1996; Philpott & Armbrecht, 2006) and ecosystem engineers (Lobry de Bruyn & Conacher, 1990; Folgarait, 1998). Their abundance, as well as diversity, should trigger ecosystem functions (Walker, 1995). Given their importance, they are an obvious choice for a biodiversity study.

Ant systematics has a long history, summarized in Brown (1955) and Bolton (2003), yet our understanding of the species-level diversity of these organisms is far from complete and especially for hyperdiverse taxa such as the genus *Pheidole*. This genus ranges worldwide, and is particularly abundant in the tropics and subtropics. According to Wilson (1976) it is one of the three most prevalent world ant genera in terms of its geographic range, species diversity and local abundance. Identification of

species rich taxa such as *Pheidole* can be challenging because; sometimes comprehensive identification (keys) literature is usually not available or is inadequate for most groups of arthropods (Brehm et al., 2008), dwindling finances and taxonomy experts and methodological issues among others (Knowlton, 1993; Jarman & Elliot, 2000; Rubinoff, 2006; Rubinoff et al., 2006; Pires & Marinoni, 2010). Nevertheless, species identification still continues either using traditional based methods especially morphological characters (Wiens & Penkrot, 2002; Ward, 2007), molecular characters (Blaxter, 2004; Floyd et al., 2002; Hebert et al., 2003*a,b*; Tautz et al., 2003) or a combination of multiple methods (Mengual et al., 2006, Smith et al., 2008).

In order to accelerate the analysis of biodiversity (Brooks et al., 2004; Smith et al., 2005) and thus increase the number of biodiversity inventories, DNA sequence data is successfully being used to test morphology-based taxonomies (Wiens & Penkrot, 2002; Smith et al., 2005; Smith et al., 2008). Congruence in biodiversity estimates when using any two or more methods in species identification is an indicator of methodological effectiveness, while inconsistency would mean the need for a further detailed examination of species in question. Furthermore, a combination of methods is likely to offer reliable biodiversity estimates and such information is of much value for conservation planning.

1.3 Phylogenetic composition and structure of ant communities in secondary tropical forests

Habitat alterations and its effects to biodiversity is currently a main challenge for community ecologists and conservation biologists. Habitat disturbance leads to biodiversity losses, thus affecting species composition in communities (Fahrig, 2003) and also changes the balance of forces acting on local communities (Dinnage, 2009). However, most studies of phylogenetic community structure especially in species rich tropical ecosystems have largely focused on plant communities (Chazdon et al., 2003; Kembel & Hubbell, 2006; Letcher, 2010), and such high attention is yet to be given to invertebrates such as ants, despite their abundance, species richness (Floren & Linsenmair, 2000) and ecological dominance (Fittkau & Klinge, 1973; Rockwood & Glander, 1979; Floren et al., 2002; Rico-Gray & Oliveira, 2007) in these ecosystems.

Furthermore, the search for patterns in the species composition, phylogenetic structure, and for the processes that cause these patterns, has seldom employed information about the phylogenetic relationships of species within those communities. Instead, in most studies, species are usually treated as equivalent units, with independent functional traits (Diamond & Case, 1986; Roughgarden, 1989; Webb & Peart, 1999; Weiher & Keddy, 1999; but see Cotgreave & Harvey, 1991).

The performances of such studies based on classical indices in highlighting the effects of both natural and anthropogenic disturbance on community composition and structure often depend on multiple factors, which could vary under different environmental settings (Sueur, 2008; Patrício et al., 2009). Most of these classical indices may provide imprecise results in the sense that taxonomy, phylogeny, and functional variability among species are not taken into account when a community is assessed (Heino et al., 2005), and this may often lead to wrong decisions on conservation prioritization. With the increased availability of molecular data and new analytical methods, several authors have therefore proposed the inclusion of phylogenetic information in community studies, with both unstandardized and standardized measures being used in evaluating community phylogenetic composition and phylogenetic community structure (Warwick & Clarke, 1995, 1998; Webb et al., 2002; Chave et al., 2007). Due to their independent nature on species richness, the standardized measures can be compared across various habitats.

Phylogenetic composition and structure of communities is influenced by several factors, which may include habitat variation, assembly rules and taxonomic scale among others. In a case of communities in recovering ecosystems, phylogenetic distinctness is likely to increase with improvement in habitat quality. Additionally, depending on how broadly or narrowly communities are defined, factors such as competition (McGlynn & Kirksey, 2000), and habitat effects (Torres, 1984; Perfecto & Vandermeer, 1996; Carvalho & Vasconcelos, 1999; Kaspari & Weiser, 2000; Oliver et al., 2000) may play a key role in determining the community assembly patterns. Studies have also observed that communities in disturbed habitats may be structured differently from those in stable undisturbed habitats depending on the acting processes (Dinnage, 2009; Verdú et al., 2009). For example, a model proposed by Weiher & Keddy (1995) predicts that the primary force on community composition under high environmental adversity will be biotic factors such as competitive

interactions. Conversely, under low levels of environmental adversity abiotic factors will be more important for community assembly. Although there have been various studies on factors influencing phylogenetic composition and structure in communities (Webb et al., 2002; Schnell et al., 2003; Cavender-Bares et al., 2006), none of these have focused on ant communities across a tropical forest successional gradient. Moreover, in as far as the application of various null models in detecting the signature of processes generating community structure (Gotelli, 2001) is concerned; most studies have tended to focus on a single model, and this may lead to false interpretation of observed community patterns.

Secondary tropical forests at different phases of recovery are ideal for testing how succession influences community composition, and also assess the processes determining how ant communities are assembled from a common species pool at different taxonomic scales. Moreover, we test how different metrics allow for the comparison and interpretation of observed community patterns. Ants being keystone species in ecosystems (Folgarait, 1998; Bihn et al., 2008) form a suitable group for this analysis and may be used to make generalizations on the recovery state of other arthropod communities in the secondary forests. Knowledge on phylogenetic composition and community structure of species is critical in identifying the habitats/sites with the highest biodiversity value and other ecologically relevant changes in the environment (Philippi et al., 1998), and thus warranting conservation priority. For instance, more phylogenetically diverse communities have more conservation value as compared to those with low phylogenetic diversity.

1.4 The study area

All ant samples and community abundance data used for the studies included in this thesis were obtained from a field work carried out in the Rio Cachoeira Nature Reserve in the Atlantic Forest of Brazil. The Atlantic Forest or *Mata Atlântica* once extended almost continuously along Brazil‘s Atlantic coast, from the northern state of Rio Grande do Norte south to Rio Grande do Sul (Figure 1), and it forms a narrow fringe of forest sandwiched between the ocean and the dry uplands of the planalto (J. Bihn Dissertation, 2008). Its flora and fauna may include 1–8% of the world’s total species (da Silva & Casteleti, 2003).This biome is known for its highest percentages of

endemism in the world, with more than 8000 of an estimated 20000 species of plants and 650 endemic vertebrates being thought to be endemic (Mittermeier et al., 2005). Examples of animals endemic to the Atlantic Forest of Brazil include: 92% of the amphibians (Lynch, 1979) and 948 of 2120 butterfly species (Brown et al., 2000) are found nowhere else in the world.

Human activities have caused immense degradation of the forests in this biome, and currently, less than 7% of the original forest cover is left. Most of the remaining Atlantic Forest exists in small fragments (<100 ha; Ranta et al., 1998) that are isolated from each other and are composed by second-growth forests in early to medium stages of succession (Viana et al., 1997; Metzger, 2000; Metzger et al., 2009, Bihn et al., 2010). The majority of the remaining patches of old-growth forests are embedded in a mosaic of secondary forests, tree plantations, pastures, and agricultural crops (Dean, 1995; Bihn et al., 2008, 2010). Brazil‘s coastal forests are considered as one of the five *hottest* biodiversity hotspots (Myers et al., 2000), due to the increased habitat loss that has left large numbers of the region‘s endemic species severely threatened with extinction (Brooks et al., 1999).

Figure 1: Original distribution of the Atlantic Forest in South America. Map adapted from *The Atlantic Forest Region Hotspot* map, © Conservation International.

1.5 Main Objectives

The objectives of my thesis included the following:

(1) Determine the diversity of a hyperdiverse ant genus *Pheidole* in Rio Cachoeira Nature Reserve found in the Atlantic Forest of Brazil using a combination of morphological (morphospecies) and molecular (MOTU) identification approaches (Chapter 2).

(2) Examine the effect of habitat perturbations/succession on phylogenetic composition and structure of ant (genus *Pheidole*) communities along the forest succession stages in tropical secondary forests of Brazil (Chapter 3).

(3) Assess the determinants of phylogenetic assembly patterns using various ant genera along the forest succession gradient in the Atlantic Forest of Brazil (Chapter 4).

1.6 Results and Discussion

Chapter 2: DNA barcodes for species identification in the hyperdiverse ant genus *Pheidole* (Formicidae: Myrmicinae)

Loss of tropical forest habitats has been identified as a major threat to biodiversity. The biodiversity value in these forests is crowded with much uncertainty because most species of small-bodied taxa are still under-described. Using species of a hyperdiverse ant genus *Pheidole* from the Rio Cachoeira Nature Reserve, we sought to establish whether the morphology-based assignment of individuals into species is supported by DNA-based species delimitation when estimating species richness. Putative species (morphospecies) were identified using morphological characters, 47 of which were used for sequencing.

We utilized a single gene (Cytochrome oxidase 1; *cox1*: Hebert *et al.,* (2003*a*, *b*) for DNA-barcoding, and noted that mean sequence divergence within same morphospecies never exceeded 2% except for one case. Molecular Taxonomic Units (MOTU: Floyd *et al.,* 2002; Blaxter *et al.,* 2005) were allocated using sequence divergence thresholds of 2% and 3%, and then matched with the morphospecies. Both thresholds yielded the same number of MOTUs, with a 79% match success. Mismatch between MOTUs with morphospecies may be due to misidentification, incomplete lineage sorting, mitochondrial introgression, or probably due to unrecognized cryptic species (Herbert *et al.,* 2005; Meyer *et al.,* 2005), suggesting taxa in need of further attention. We observed very low success when matching our MOTUs with *Pheidole* species already in a *CO1* library, because of many undescribed ant species in the neo-tropical region, and most of those described are not occurring in the Genbank. In addition, many species in the Mata Atlantica may be endemic (R. Brandl, personal communication). Our findings demonstrated that diversity estimates using *CO1* MOTU together with morphological taxonomy offers a means to map the occurrence of ant species that still wait to be formally described and included into keys for identification.

Chapter 3: Phylogenetic composition and community structure of ant genus *Pheidole* along forest succession gradient.

This chapter compares the patterns of phylogenetic composition/relatedness and the processes structuring leaf litter ants (genus *Pheidole*) in 11 study sites scattered across the forest succession stages in the Rio Cachoeira Nature Reserve (in the Atlantic Forest of Brazil). We used an unstandardized index– Phylogenetic distinctness (Δ*), and standardized indices sesPD, NRI and NTI, under various constrained and unconstrained null models. Δ* showed sensitivity to habitat variation/succession (see Desrochers & Anand, 2004), and was strongly and positively correlated to species richness as opposed to the three standardized measures. Δ* increasing trend along the forest succession stages implies that the recovery of the secondary forests is accompanied by an increase in phylogenetic distinctness and species richness in this genus (see also Bihn et al., 2008). Δ* index showed some tendency to saturate at various sites, with some site having same values, and his may be influenced by sample size (see Warwick & Clarke, 1995; Izsak & Price, 2001).

Tests on the phylogenetic patterns along the succession gradient revealed that on overall, both the unconstrained and the constrained null models lead to phylogenetically clustered communities. This indicates that *Pheidole* species occurring together in each site are more closely phylogenetically related than expected by chance (see Webb, 2000; Kembel & Hubbell, 2006). Clustering of ant communities indicates that habitat-use is a conserved trait within the pool of species in the community; interpreted as evidence of habitat selection for ecologically similar, phylogenetically related species (see Webb, 2000; Webb et al., 2002; Cavender- Bares et al., 2004). This may imply occurrence of ecologically more similar environments (spatial heterogeneity) along the forest gradient in terms of resource availability and habitat conditions (see Bihn et al., in prep). Based on our findings, old-growth forests in Brazilian Atlantic forest deserve higher conservation priority since they are more phylogenetically diverse compared to other forest succession stages (see also Bihn et al., 2008, 2010).

Chapter 4: Phylogenetic community structure of ant genera in secondary tropical forests of Brazil

We examined the phylogenetic community structure using a genus level phylogeny to assess if ant communities differ from random expectations. To measure the phylogenetic community structure, we used sesPD, NRI and NTI indices. We observed that the assembly patterns across the sites depended on the metric used, although there were common patterns in some sites based on the three metrics. This may be attributed to sensitivity of metrics to different aspects of community structure (see Webb, 2000; Vamosi et al., 2009). Forest succession stages seemed to influence the observed phylogenetic patterns as reflected by the hump-like distribution patterns of community structure, which may imply that resources for different genera were not distributed in a heterogeneous manner along the forest succession stages. Communities showed both over-dispersion and clustering across sites and this variation may be also attributed to the effects of the null model used in this study. However, on average the ant communities were phylogenetically over-dispersed, meaning that competitive interactions play a dominant role in structuring ant genera communities in the secondary tropical forests. On the contrary, we noted that environmental filtering is the main process in structuring ants at species level as demonstrated using species in the genus *Pheidole* (Chapter 3). This indicates the influence of taxonomic scale/species composition in habitats on community structure. (see Webb et al., 2002 ; Cavender-Bares et al., 2006).

1.7 Conclusions and recommendations

In this thesis, we estimated the diversity of a hyperdiverse ant genus, the phylogenetic composition and structure of ant communities as well as the processes responsible for these community patterns in Rio Cachoeira Nature Reserve, within the Atlantic Forest of Brazil. Several major conclusions from this study were:

1. Species identification using DNA-barcoding together with morphological taxonomy can be complementarily used to estimate diversity in hyperdiverse taxa as *Pheidole*.

2. Succession influences the phylogenetic composition of ant communities, and on overall, old growth forests are the most phylogenetically diverse habitats, thus warranting high conservation priority.

3. Both environmental filtering and competitive interactions play a role in structuring litter-ant communities at different taxonomic scales in the secondary tropical forests of Brazil.

As per the results, we recommend that distinct mitochondrial lineages within morphological species in the genus *Pheidole* require further detailed genetic and morphological studies, or a combination of other methods. In regard to community patterns, future phylogenetic community studies should measure the traits for the organisms and also the environmental variables in the habitat, to allow for better interpretation of ecological processes structuring ant communities and the resultant patterns. Finally, conservation efforts need be largely concentrated in the phylogenetically diverse old growth forest remnants to ensure maximum biodiversity is preserved. As a step to achieving this, the local population should be educated on conservation issues so that further forest encroachment is halted and thereby allowing for recovery and minimised losses of biodiversity in the secondary forests**.**

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Chapter 2

2 DNA barcodes for species identification in the hyperdiverse ant genus *Pheidole* (Formicidae: Myrmicinae)

2.1 Abstract

DNA sequencing is increasingly being used to assist in species identification in order to overcome taxonomic impediment. However, few studies attempt to compare the results of these molecular studies with a more traditional species delineation approach based on morphological characters. We sequenced the mtDNA Cytochrome oxidase subunit 1 (*CO1*) gene, measuring 636 base pairs, from 47 ants of the genus *Pheidole* (Formicidae: Myrmicinae) collected in the Brazilian Atlantic Forest to test whether the morphology-based assignment of individuals into species is supported by DNA-based species delimitation. 20 morphospecies were identified, whereas the barcoding analysis identified 19 Molecular Operational Taxonomic Unit(s) (MOTUs). We found that 15 out of the 19 DNA-based clusters allocated using sequence divergence thresholds of 2% and 3%, matched with morphospecies. Both thresholds yielded the same number of MOTUs. Only one MOTU was successfully identified to species level using the *CO1* sequences of *Pheidole* species already in the Genbank. The average pairwise sequence divergence for all 47 sequences was 19%, ranging between 0-25%. In some cases, however, morphology and molecular based methods differed in their assignment of individuals to morphospecies or MOTUs. The occurrence of distinct mitochondrial lineages within morphological species highlight groups for further detailed genetic and morphological studies and therefore we advocate a pluralistic approach using several methods to understand the taxonomy of difficult lineages.

Key words *CO1,* DNA-barcoding, morphospecies, MOTU, Taxonomy

2.2 Introduction

Identification of species can be difficult, often requiring specialized knowledge and thereby representing a limiting factor in biodiversity inventories (Monaghan et al., 2005). Therefore, based on the growing concern over the threats to biodiversity, recent publications have emphasized the need to accelerate the analysis of biodiversity (Brooks et al., 2004; Smith et al., 2005) either by using morphospecies (Hammond, 1994; Oliver & Beattie, 1996; Barratt et al., 2003; Krell, 2004) or by using DNA-based methods (Blaxter, 2004; Floyd et al., 2002; Hebert et al., 2003a; Tautz et al., 2003). Both, morphological and molecular approaches have been faced with criticism (Pires & Marinoni, 2010) due to the deficiencies encountered when using only a single approach for species identification (Knowlton, 1993; Jarman & Elliot, 2000; Rubinoff, 2006; Rubinoff et al., 2006). The comparison of results obtained by various approaches can aid in overcoming methodological issues in species identification (Mengual et al., 2006; Smith et al., 2008). A further advantage of integrating molecular and morphological approaches (Dayrat, 2005; Cardoso et al., 2009) is that it promotes taxonomic stability (Padial et al., 2010).

In this paper, we used a single gene (Cytochrome oxidase subunit 1; *CO1*) as proposed by Hebert et al. (2003a & b) for barcoding, using morphologically pre-defined species (morphosecies) of the hyperdiverse ant genus *Pheidole* (Formicidae: Myrmicinae). We evaluated how DNA barcoding enables the definition of Molecular Taxonomic Units (MOTU; Floyd et al., 2002, Blaxter et al., 2005), and linked the delineated MOTUs to the morphospecies in order to assess congruence success. Our study focused on *Pheidole* samples from a region in the Brazilian Atlantic Forest because it is considered as one of the “hottest hotspots” of biodiversity (Myers et al., 2000).

2.3 Materials and Methods

Research Area.

Specimens for this study were obtained between June and September 2003 from a survey conducted in 27 sites across the Rio Cachoeira Nature Reserve (25°18´51"S, 48°41´45"W). The reserve is located near the city of Antonina, in the coastal region of

the Brazilian state of Paranà. The landscape varies from littoral plains with isolated hills to the uplands of the Serra do Mar mountain range. Lowland and submontane forests originally covered this area, but these dense ombrophilous forests have been intensely exploited. Old growth forests remain only in the hillside regions. The resulting landscape mosaic consists of old growth forests and secondary forests in various stages of succession and pastures (Bihn et al., 2008, 2010).

Definition of Morphospecies.

Pheidole specimens were identified to species with the key for neo-tropical species given in Wilson (2003). In cases where identification was not possible with this identification key (e.g. when major workers were not collected) or led to ambiguous results, ants were sorted into morphospecies by J.H.B. using characters described in Wilson (2003). Additionally, morphometric measurements were made to aid in the assignment of specimens into morphospecies (for details on the set of measurements taken and their definition, see Longino 2008).

DNA Extraction, Amplification and Sequencing.

Field collections were preserved in 95% EtOH until time for DNA extraction. Specimens already examined and identified to be *Pheidole* morphospecies using morphological taxonomy by J.H.B. were used for DNA extraction. mtDNA was isolated for at least two workers from each morphospecies using the Qiagen DNeasy tissue extraction kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. In cases where rare species were involved, DNA was extracted from a single individual in which case either two legs or the whole individual was used. When we found deep genetic divergences within a supposed morphspecies, more specimens were sequenced to provide a better understanding of the distribution of the genetic variation in relation to morphology.

Polymerase chain reaction (PCR) was conducted under the following reaction volumes: 2–4 µl DNA template, 2 µl in 10× PCR buffer, 1.6 µl of dNTPs in 10 mM concentration, 1 µl of each primer in 10 mM concentration, 0.2 µl of Taq DNA polymerase, and distilled water for a total reaction volume of 20 µl. Reactions conditions included: initial denaturation at 95ºC for 5 min; 33 cycles at 95ºC (30 s), 45–52ºC at 40–48 s (annealing time and temperature depended on primer used), and

72ºC at 1 min; and a final elongation at 72ºC for 10 min Reactions were done using an Eppendorf Thermal Cycler. Full length sequences were amplified using primer pair LCO1490–GGTCAACAAATCAAAAGATATTGG and HCO2198–TAAACTTTCAGGGTGACCAAAAAATCA (Folmer et al., 1994). Primer pair LF1–ATTCAACCAATCATAAAGATATTGG and LR1–TGATTTTTTGGACATCCAGAAGTTTA (Herbert et al., 2004a) was also used on specimens that were difficult to amplify using primers HCO/LCO. The two primer pairs gave the same length of base pairs. Products were visualized on a 2% agarose Gel and samples containing clean single bands purified using QIAquick PCR purification kit (Qiagen, Hilden, Gernany). The purified samples were sent for sequencing (AGOWA genomics, Germany), whereby the primers used in each case for amplification served as sequencing primers. All samples were sequenced in both directions and the obtained sequences aligned using BioEdit version 7.0.9.0 (Hall, 1999). The resultant fragments were approximately 658 base pairs (bp), and were identified as *CO1* fragments for the ant genus *Pheidole,* with BLAST procedure search in GenBank (Altschul et al., 1997) done between the 2008 and 2009. After trimming, the aligned sequences were 636 bp long and free from gaps. A translation with the invertebrate mitochondrial code returned uninterrupted amino acid sequences. These observations support the conclusion that the sequences we analysed were mitochondrial DNA and not nuclear pseudogenes (Bensasson et al., 2001).

Phylogenetic Analysis

Sequence divergences were calculated using the Kimura two parameter (K2P) distance model (Kimura, 1980) and the relationships between sequences was in a first step visualized by a Neighbor-Joining (NJ) tree (Saitou & Nei, 1987) using version 4 of MEGA software (Tamura et al., 2007). To infer the phylogenetic relationships among the supposed morphospecies of *Pheidole*, phylogenetic analyses were performed using MrBayes version 3.1.1 (Huelsenbeck & Ronquist, 2001), using General Time Reversible (GTR) model. Bayesian posterior probabilities (bpp) were estimated as the proportion of the trees sampled after the burn-in that contained each of the observed bipartitions (Rannala & Yang, 1996; Larget & Simon, 1999). The phylogenetic tree was rooted using two species of the tribe Pheidolini (*Aphaenogaster texana* and *Messor julianus*).

The MOTU delineation from the *CO1* sequences relied on two aspects: (1) Individuals were considered to be the same MOTU if sequences from the same morphospecies clustered together in the phylogenetic tree. A MOTU was thus defined as least inclusive terminal groups (i.e. closest to the tips). (2) Sequence clusters with a mean divergence value less than or equal to a threshold of 2% and 3% as proposed by Herbert et al. (2004b), were considered as MOTUs. In this case, if sequences from two different morphospecies formed the same cluster, they only qualified to be a single MOTU if their mean sequence divergence was below or equal to thresholds 2 and 3%.

We further examined match success of the 47 sequences in relation to *CO1* sequence of species in the genus *Pheidole* already present in the *CO1* Genbank library (NCBI, GenBank; http://www.ncbi.nlm.nih.gov/) (searches done between 2008 and 2009). In cases where the match success was above 95%, the species name for that MOTU was allocated. To establish the distribution of genetic divergence and positioning of our MOTUs in relation to *Pheidole* species from other regions, all *CO1* sequences (genus *Pheidole)* which contained 640 base pairs and above (sequences retrieved on 2 March 2011) were extracted from the Genbank. A total of 141 sequences were obtained and combined with 47 sequences from this study for further alignment. The final set of 188 sequences was trimmed to 636 base pairs, and using K2P distance (Kimura, 1980), a histogram and a Neighbor-Joining (NJ) tree were constructed. This was implemented in package ape (Paradis et al., 2004) available in R (R Development Core Team, 2009); tree in Figure 3).

2.4 Results

This study produced a final aligned 636 bp fragment characterized with no gaps for all the 47 sequences. Sequences were heavily AT biased (in our case especially in the 3rd codon position), as expected in insect mtDNA (Crozier & Crozier, 1993; Table 1). The average pairwise sequence divergence of all 47 sequences is 19%, ranging from 0-25% (Figure 1a). The distribution of K2P distances for 47 sequences showed one peak near zero and another between 18-24%, while the 141 *CO1* sequences from Genbank had a peak between 16-24% of sequence divergence (Figure 1b).

Through morphology-based taxonomy 20 morphospecies were identified, three of which were allocated species names (Figure 2). DNA analysis identified 19

MOTUs, 15 of which matched with the morphospecies – about 79% match success. 46 sequences showed a matching of below 95% with *CO1* sequences from the Genbank, which ranged from 83% to 87%. Only sequence for morphospecies JHB14 showed a 96% match with Genbank sequence of *Pheidole laticornis*. A phylogeny containing a combination of 141 *CO1* sequences of *Pheidole* species from the Genbank and our 47 sequences showed distinct clusters for the MOTUs (Figure 3, taxa in blue). Specimens with only one sequence were regarded as a MOTU using the 2 or 3% criterion. In four MOTUs morphological taxonomy did not match results of the DNA-based approach.

The clustering of the 47 *CO1* sequences in NJ and Bayesian trees showed congruence with most morphospecies groups, with most nodes immediately below (i.e. defining) clusters showing a bootstrap support and a posterior probability of 100 (Figure 2). Divergences between sequences making up different *CO1* clusters (MOTUs) were far higher than divergences within a cluster of MOTUs (11-fold higher), with average Kimura-2-Parameter (K-2-P) divergences within and between clusters being 1.8% and 20% respectively (Figure 1). Exceptions occurred where deep sequence divergences were apparent between individuals identified as the same morphospecies (the two red bars in Figure 2 (JHB03285G01 & JHB01425G01). These have a mean sequence divergence of 12.6%; a divergence 6–4 fold higher than our 2 and 3% thresholds respectively, which were used to allocate individuals into their respective clusters.

Table 1. Sequence statistics for the 47 specimens used in the analysis of ant genus *Pheidole.*

			Average (Avg)					
Domain	Identical pairs	Transitional pairs (si)	Transversional pairs (sv)	R (si/sv)	T	C	A	G
Avg	528	55	52	1.1	37.9	20.8	28.9	12.4
1st position	192	14	6	2.1	25.6	19.3	34.3	20.8
2nd position	210	1	1	0.6	44.8	24.6	16.0	14.7
3rd position	127	41	44	0.9	43.4	18.4	36.5	1.7

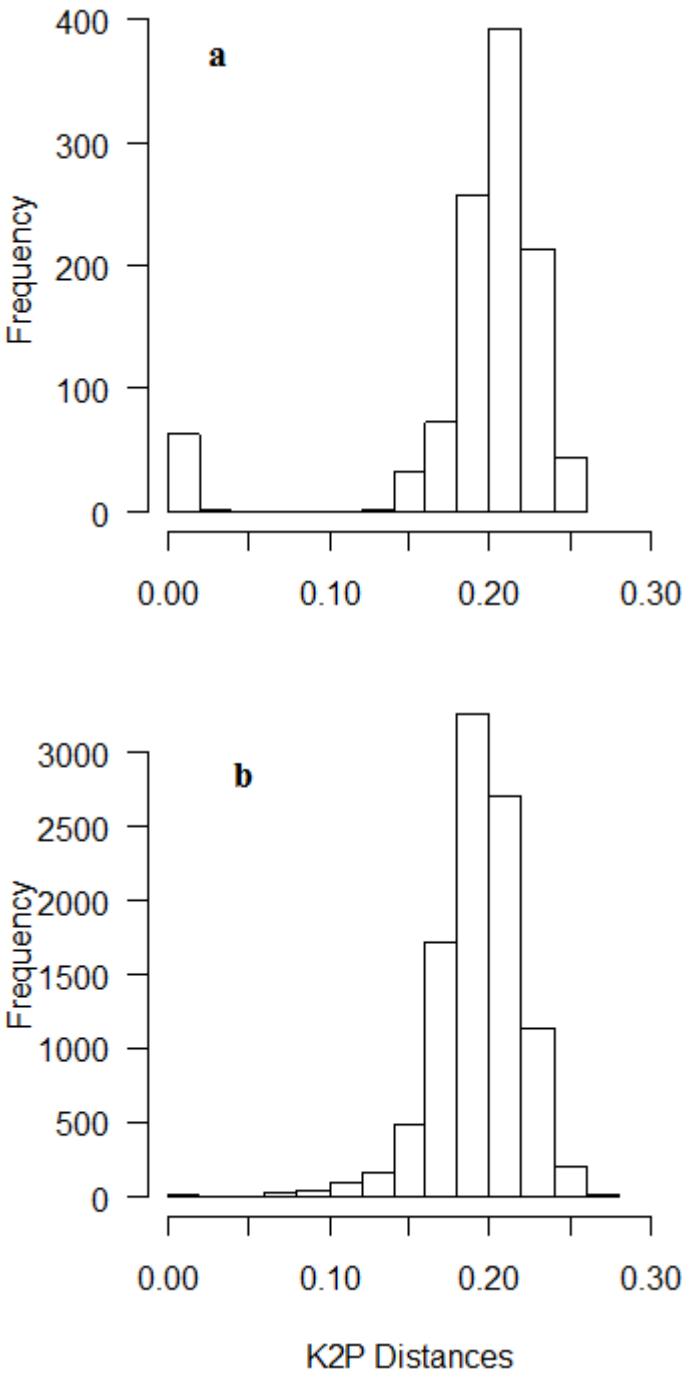

Figure 1. Distribution of pairwise distances of ant genus *Pheidole* calculated using Kimura two-parameter model (Kimura, 1980). (a) Among 47 Cytochrome oxidase 1 (*CO1*) sequences from Rio Cachoeira Nature Reserve in Brazil. (b) Among 141 *CO1* sequences from the Genbank. The 47 sequences have a peak near zero and another between 18% –24% while the 141 sequences have a major peak between 16% –24% of sequence divergence.

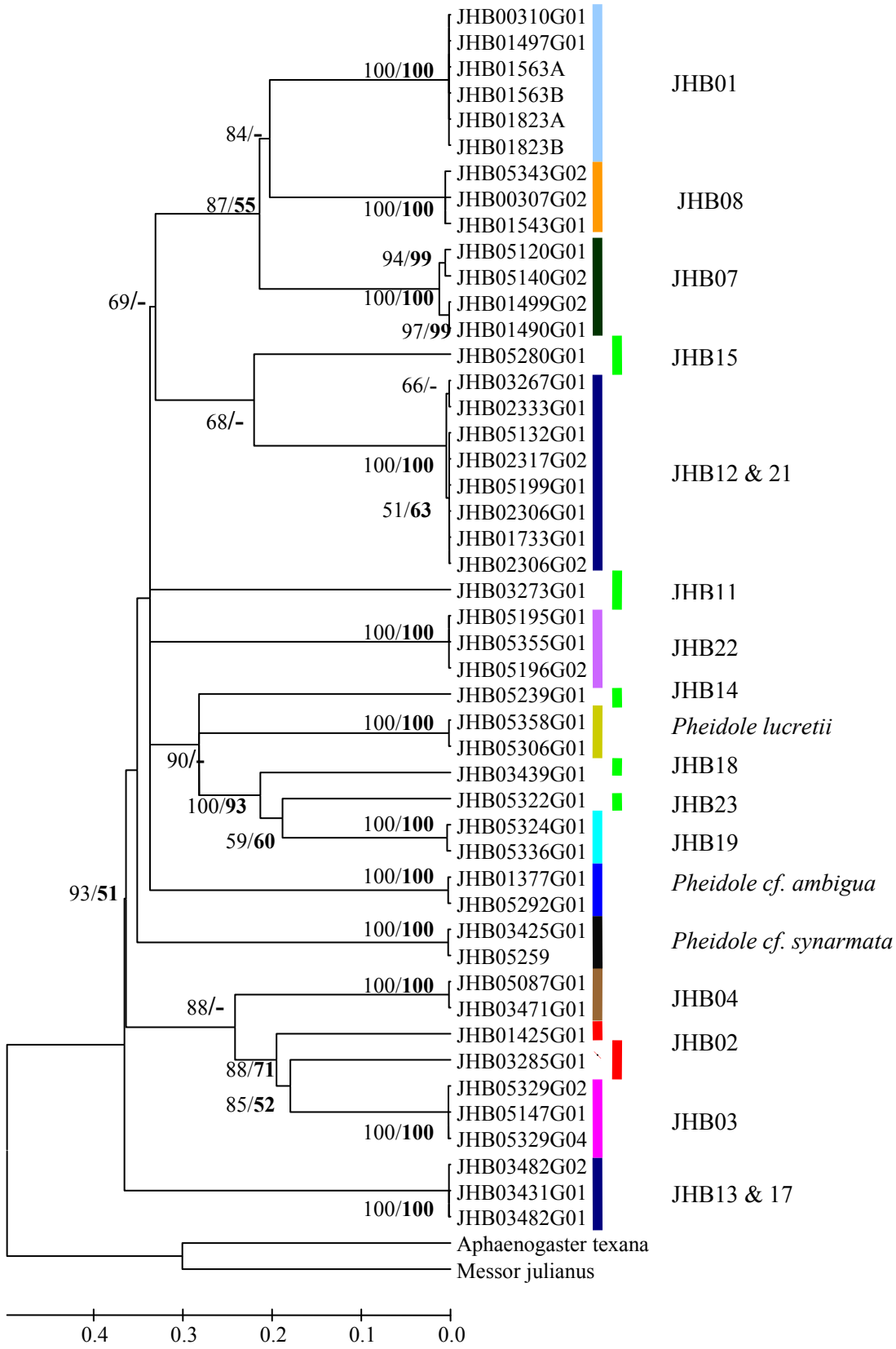

Figure 2. Linearized Bayesian tree of ant genus *Pheidole* from Rio Cachoeira Nature Reserve in Brazilian Atlantic Forest which defines 19 MOTUs. The clustering of individual sequences in the tree indicates the membership of each MOTU. MOTU were

inferred from a tree dependent clustering process, coupled with thresholds 2% and 3%. Each coloured bar represent a MOTU. The 5 green bars represent cases where only one individual was sequenced, 2 red bars indicate possible cryptic taxa and the 2 dark blue bars indicate MOTU with shared taxa. The three names in front of the bars represent *Pheidole* species assigned names based on morphological taxonomy and the numbers preceded by JHB represent the different morphospecies. Posterior probability values for Bayesian tree and bootstrap support values for NJ tree (in bold) above 50 % are indicated on the nodes. Dash (-) indicate bootstrap values below 50.

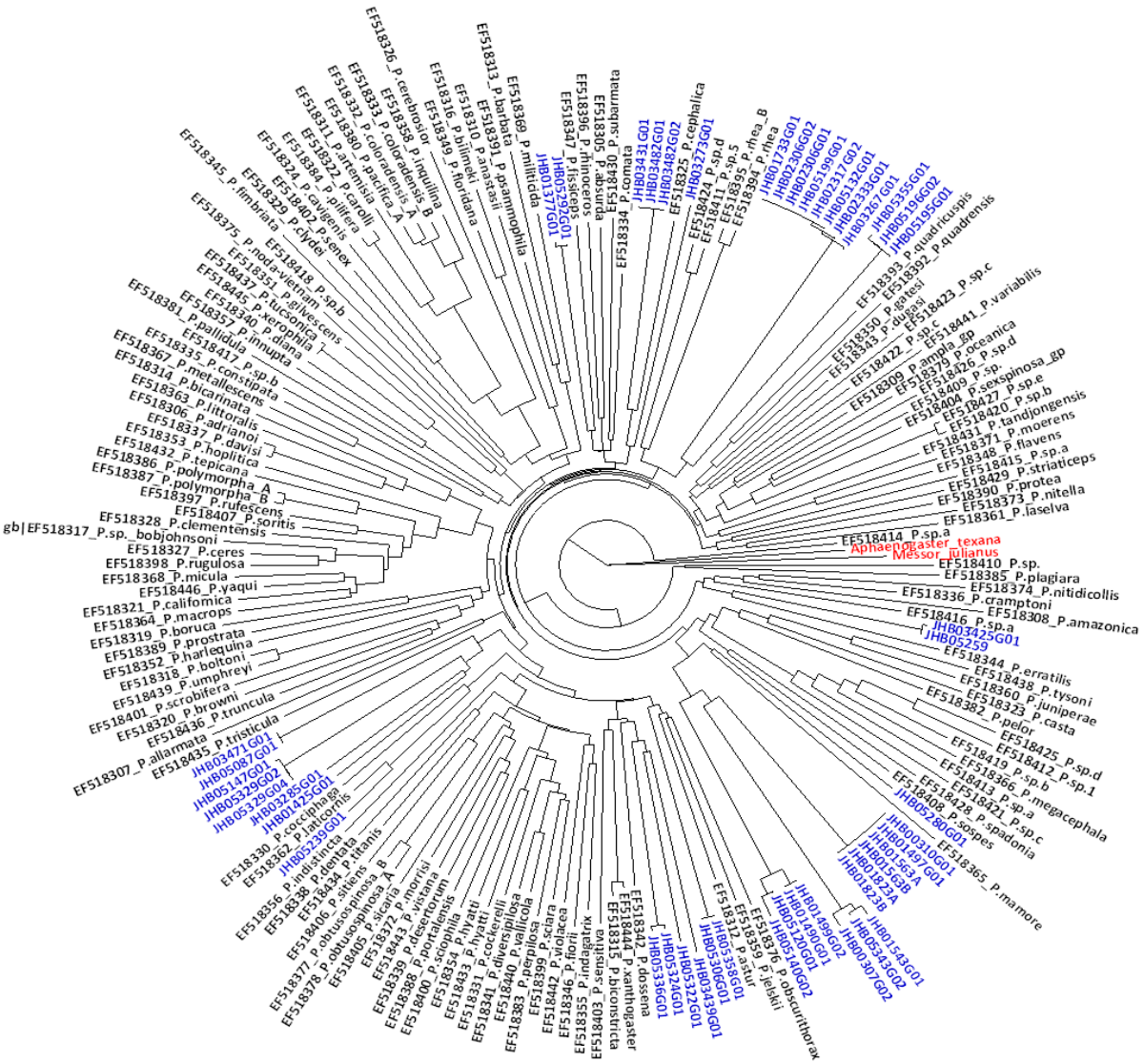

Figure 3. A combined phylogeny of *CO1* sequences all from the genus *Pheidole* from the Genbank and 47 sequences (taxa in blue) from Rio Cachoeira Nature Reserve (RCNR). The 47 sequences formed distinct clusters in relation to those from the Genbank. The tree is rooted using the taxa in red.

2.5 Discussion

Our results indicate that *CO1* sequences show promising success in allocating morphologically pre-defined individuals into distinct *Pheidole* MOTUs. Most sequences clustered into cohesive, well-differentiated groups, most of which show congruence with the morphospecies defined by an experienced taxonomist. The majority of nodes immediately defining the clusters showed remarkably high levels of nodal support. Furthermore, most clusters remained distinct as sample sizes increased during the progress of our work, an indication that such groups included distinct *CO1* lineages rather than scattered sequence variation (Hajibabaei et al., 2006). In addition, sequence divergence in *CO1* mtDNA within MOTU (clusters) was usually much lower than 2%, whereas divergence between the clusters was often greater, but remained within the range of divergences between *CO1* sequences of *Pheidole* species from the Genbank. This is in general agreement with empirical levels of divergence found between species in barcoding studies (Hebert et al., 2003b). These aspects strengthen the fact that most of the identified morphospecies were indeed distinct lineages (Wiens & Penkrot, 2002).

A total of 20 morphospecies had been recovered using morphological characters whereas 19 MOTUs were recovered using barcodes. Our diversity estimate (MOTU) using threshold values of 2% and 3% were similar to the diversity estimate based on morphological characters. In cases where the 2–3% MOTU and the morphological estimation of a species differed, either different morphospecies clustered to form the same MOTU (e.g. as was the case with the two MOTUs represented by the dark blue bars in Figure 2) or sequences from the same morphospecies showed deep sequence divergence (e.g. MOTUs represented by two red bars in Figure 2) and were thus allocated as different MOTUs.

Morphological re-examination of the two MOTUs which shared morphospecies (i.e. JHB13 and JHB17, JHB12 and JHB21; MOTUs indicated with dark blue bars in Fig. 2) revealed significant differences in morphological characters between the two species. The grouping into one MOTU may be due to incomplete lineage sorting or even mitochondrial introgression (Herbert & Gregory, 2005; Meyer & Paulay, 2005). Incomplete lineage sorting or gene introgression could be possible in taxa with

shared sequences/haplotypes because, the species occurred in the same locality. This is further reflected by their low mean sequence divergence (below 0.02) and high bootstrap support for their respective clusters. Morphological variation may reflect that speciation occurs without morphological change, thus resulting in morphologically cryptic species (Adams et al., 2009).On the contrary the two MOTUs representing possible cryptic taxa (two red bars; JHB02 in Figure 2) had high mean sequence divergence and their clustering was not strongly supported, thereby qualifying to be possible different species. Further reevaluation of the four MOTUs either for introgression or cryptic diversity using mtDNA was however hampered by the limited samples.

Our findings further revealed a very low success when matching our MOTUs with *Pheidole* species already in a *CO1* library. Clusters of the MOTUs remained stable within the phylogeny even after integrating our 47 sequences with those from the Genbank. In four cases, MOTUs formed monophyletic clusters with the species from the Genbank (Figure 3). However, only in a single case the species name for the MOTU was allocated (JHB05239G01– *Pheidole laticornis*) based on the set criteria of allocating species names in this study and others (Meier et al., 2006). On overall, it was difficult to allocate species names to our MOTUs based on *CO1* sequences in the library. Our finding does not imply that *CO1* cannot be used in species identification as barcodes, but for the MOTUs in this study, other strategies will be necessary. The low success when matching our MOTUs with Genbank sequences is likely so because, only few of the more than 600 described species of *Pheidole* are included in Genbank. In addition, there are many undescribed ant species in the neo-tropical region, and many species in the Mata Atlantica may be endemic (R. Brandl, personal communication).

We did not achieve very high success in terms of congruence between the two species identification approaches, which may be attributed to the criteria we applied in delimiting MOTUs. For instance, threshold approach is vulnerable to both false positives and false negatives (Meyer & Paulay, 2005). Regardless of such shortcomings in both morphological taxonomy and DNA barcoding (DeSalle et al., 2005; Pires & Marinoni, 2010), this does not compromise their effective use for species identification (Smith et al., 2005); on the contrary, either approach help to illuminate taxonomic assignments in need of further scrutiny (Herbert & Gregory, 2005; Padial et al., 2010). Such scenarios call for a more thorough morphological and

CO1 diversity survey among the members of the involved taxa. Moreover, in cases of introgression, the analysis of a rapidly evolving nuclear sequence, such as the internal transcribed spacer region of the ribosomal repeat, will aid taxonomic resolution (Herbert et al., 2003a). However, in our study we did not manage to employ other molecular markers for species delimitation, and were only limited to mtDNA.

Due to our sampling techniques, five rare morphospecies were represented with only a single sequence (MOTUs represented by the green bars in Fig. 2) and were coded by the 2 and 3% criterion as MOTUs. A previous study on DNA barcoding of ants using these thresholds (Smith et al., 2005) recommended that it will only be by sampling multiple individuals from supposed species, or MOTUs, that inter-specific variation will be properly assessed. Otherwise it is impossible to test the hypothesis of species-level monophyly (Funk & Omland, 2003) and could lead to biodiversity overestimation. This is a valid concern in an analysis of MOTUs from inventories of hyperdiverse groups such as ants, which often include many taxa known only from single individuals (Fisher, 1999; Longino et al., 2002). Morphological identification of such rare species also calls for use of multiple individuals in order to assess the conformity in taxonomic characters within individuals of a given taxa. With additional inventories in the future, many of these rare species will be represented in collections by more specimens.

Conclusion

The aim of this study was to investigate the efficacy of DNA barcoding in delimiting pre-defined species. Our results provides an example of complementarity with which DNA barcoding can be applied together with a more conventional morphological approach, without competing or replacing the latter approach (Hebert & Gregory, 2005). Moreover, thresholds 2 and 3% proved to be effective in delineating species in the genus *Pheidole.* Since species boundaries are too complex to be only described by morphological characters or sequence divergence from mtDNA (Green, 1996; Gregory, 2005; Smith et al., 2005; Puorto et al., 2001), we propose a combination of DNA sequence data from different gene regions, in order to achieve a more accurate representation of species boundaries and biodiversity. Despite the shortcoming in match success, we demonstrated that diversity estimates using *CO1* MOTU together

with morphological taxonomy offers a means to map the occurrence of ant species that still wait to be formally described and included into keys for identification.

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Chapter 3

3 Phylogenetic composition and community structure of ant genus *Pheidole* along forest succession gradient

3.1 Abstract

The assessment of the effects of habitat variation on phylogenetic composition and the processes structuring communities is of major interest to community and conservation ecologists. In this study, we used ants in the genus *Pheidole* in evaluating the phylogenetic composition/structure and processes structuring communities along a succession gradient in the secondary forests of Brazil. Since sampling settings can influence the performance of most indices, we used standardized measures (sesPD, NRI and NTI) besides an unstandardized measure i.e. phylogenetic distinctness (Δ* – a measure of pure phylogenetic relatedness), to test their response in detecting phylogenetic community composition and structure. We also applied null models on standardized measures in order to detect potential processes generating community patterns. *Pheidole* ant communities showed sensitivity to habitat variation portrayed by the overall increasing trend in phylogenetic distinctness (Δ*) along the stages of forest succession. Δ* was strongly and significantly correlated to species richness as opposed to NTI which didn't show correlation. On overall, ant communities were phylogenetically clustered regardless of the null model used, suggesting that habitat filtering is the dominant process structuring the ant communities. That *Pheidole* ant communities in our study likely form non-interactive assembly, is a notion which contrasts with many studies suggesting that competitive interactions structure closely related ant communities. The observed high phylogenetic diversity in old growth forests compared to other forest stages reflects the conservation importance that need to be attached to these habitats in ensuring that maximum biodiversity value is preserved.

Key words: Community structure, null models, succession, phylogenetic composition.

3.2 Introduction

Globally, there is an increasing trend in degradation of natural ecosystems (Turner & Corlett, 1996; Didham, *et al.,* 1996; Wardle, 1999), and the species rich tropical forests have not been an exception. Degradation of habitats does not only reduce biodiversity but also has drastic consequences on species composition and the mechanisms structuring these communities (Floren & Linsenmair, 1999, 2000, 2001). This resulted in efforts exploring the changes in phylogenetic composition and structure of communities during succession, following ecosystem disturbance (e.g. Dinnage, 2009; Bihn et al., in prep.).

Ants (Hymenoptera: Formicidae) are useful taxa for the study of phylogenetic composition and community patterns in secondary tropical forests. The strong interactions between ants and other organisms make the former important —keystone species in tropical forests. Furthermore, ants structure their environment through their roles as seed dispersers (Beattie, 1985; Levey & Byrne, 1993), predators (Andersen, 1992; Kaspari, 1996a; Philpott & Armbrecht, 2006) and ecosystem engineers (Lobry de Bruyn & Conacher, 1990; Folgarait, 1998). In particular, leaf litter ants are considered to be useful indicators of ecosystem disturbance in various habitats (Majer et al., 1984; Agosti et al., 2000) and have been found to show congruent patterns in their responses to environmental change with other taxa (Lawton et al., 1998; Alonso, 2000). Moreover, litter ants play a major role in ecological functions such as decomposition, and this means that the phylogenetic composition and patterns of ant communities directly influence ecological functions (Krushelnycky & Gillespie, 2008; Lessard *et al.,* 2009). Therefore, studying phylogenetic composition and structure of litter ant communities will not only broaden our understanding on ant ecology during succession in secondary forests, but is also an important guide to current efforts of biodiversity conservation.

A major challenge in studies of successional dynamics in tropical forests is to reveal the relative importance of deterministic versus stochastic processes affecting species richness, phylogenetic composition and community patterns (Floren & Linsenmair, 2001). For instance, resource availability and habitat heterogeneity have been reported as important factors driving ant species composition and community

patterns in tropical ecosystems (Bestelmeyer & Wiens, 2001; Ribas *et al*., 2003; Armbrecht *et al*., 2004; Ribas & Schoereder, 2007; Bihn et al., in prep). In a case of secondary tropical forests, we expect that, as environmental adversity decreases and resources increase from early successional stages to late successional stages, more ant species will be added to the habitats either through colonization or by chance, and this may translate to increase in phylogenetic diversity and distinctness along the forest succession gradient. Additionally, since the nature of phylogenetic community composition influences the phylogenetic structure of communities, we expect that ant species whose functional traits are conserved, are likely to share habitat tolerances and preferences, thus resulting to their coexistence due to phylogenetic attraction or competitive exclusion will limit their coexistence due to phylogenetic repulsion (see also Webb et al., 2002; Cavender-Bares et al., 2004; Ulrich, 2004; Losos, 2008).

In an attempt to understand dynamics in species composition and community patterns, recent studies have emphasized the adoption of biodiversity indices that take into account phylogenetic information of species (Webb et al., 2002; Chave et al., 2007). Several researchers have argued that the taxonomy, phylogeny, and functional variability among species need to be taken into account especially when assessing phylogenetic composition and structure of communities, and their response to habitat dynamics (Warwick & Clarke, 1995, 1998; Anu & Sabu, 2006; Slingsby & Verboom, 2006; Helmus et al., 2007; Kraft et al., 2007; Swenson et al., 2007; Tolimieri & Anderson, 2010; Bevilacqua et al., 2011). Since some of these measures are independent from the number of species, this makes them independent of sample size and sampling efforts and, thus, potentially usable over a broad range of environmental contexts, even when analyzing historical (Cusson et al., 2007) and simple presence–absence data (Price et al., 2006). In the analysis of phylogenetic community patterns, null models have been used to detect the signature of certain processes like species interactions or niche sorting (Gotelli, 2001). However, many phylogenetic community studies tend to concentrate on a single null model and therefore little is known about how using different null models influence results of the analyses of community patterns.

In this study we focus on ants in the genus *Pheidole* from secondary tropical forests of Brazil with the aim to establish their phylogenetic composition and community structure along the forest succesional gradient. Due to its hyperdiverse

nature in these tropical forests (Bihn et al., 2008), the genus *Pheidole* is a good model for this study and can therefore be used to make further interpretations on the response of animal biodiversity to forest regeneration after disturbance. Several studies have been done in regard to composition and community structure of ants (Hölldobler & Wilson, 1990; Fournier et al., 2002; Fowler et al., 2000; Yanoviak & Kaspari, 2000; Floren et al., 2001; Gotelli & Ellison, 2002; Bihn et al., 2010). Although some of these have used phylogenetic approaches in establishing the community structure of ants, the influence of aspects such as succession/habitat disturbance on patterns of phylogenetic relatedness and phylogenetic community assembly of ants in tropical forests are yet to be addressed. By using a species level phylogeny and community abundance data, we sought to answer the following questions: (1) What is the relationship between the indices used and species richness? (2) Does succession influence phylogenetic composition of ant communities? (3) How are *Pheidole* ant communities phylogeneticaly structured? (4) Are the conclusions consistent when using different null models?

3.3 Materials and methods

Study area

Rio Cachoeira Nature Reserve (RCNR) (25°18′51″ S, 48°41′ 45″ W) is located near the city of Antonina, in the coastal region of the Brazilian state of Paraná. The reserve covers ~12000 ha and is a protected area owned and managed by the Sociedade de Pesquisa em Vida Selvagema e Educaçã Ambiental (SPVS). The altitude ranges from 0 to 600 m a.s.l, with mean temperatures between 16.2 °C in July and 24.5 °C in February and a precipitation of 2580 mm per year. The dense lowland and submontane forests that originally covered the area have suffered immense exploitation with most parts being turned into pastures (Bihn et al., 2008, 2010). Old–growth forests and secondary forests in various succession stages now occur in this landscape (Ferretti & Britez, 2006).

Sampling of ant data

Leaf litter ants in 240 one-square-meter quadrats distributed among 12 study sites across the reserve were sampled between June and September 2003. The sites were

selected to represent four stages of secondary forest succession, whose ages were: very young secondary forest (4-6 years), young secondary forest (10-15 years), old secondary forest (35-50 years), and old-growth forest (> 100 years; Bihn *et al.,* 2008, 2010). There were three replicates (sites) for each succession stage and the replicated sites of a particular succession stage were separated by a mean distance of 4 km (range=1-6 km). At each study site two 50 m transects (parallel, separated by 20 m) were established and leaf litter samples collected (1 m^2) at 5 m intervals along these transects (10 sampling points for each transect). This resulted in 20 samples for each site. For more details on sampling methodology see Bihn et al. (2008, 2010).

Phylogenetic relatedness measure

We used one phylogenetic relatedness index which was calculated based on the framework of Warwick & Clarke (1995) and Clarke & Warwick (1998) with the function *taxondive* implemented in the R package vegan (Oksanen et al., 2009). Phylogenetic (taxonomic) distinctness (Δ*) is defined as the average path length between two randomly chosen individuals, conditional on them being from different species (Clarke & Warwick, 1998; Rogers et al., 1999). It was calculated using the equation below.

$$\Delta^* = [\sum\sum_{i<j} \omega_{ij} x_i x_j]/[\sum\sum_{i<j} x_i x_j]$$

where ω is the distance between species i and j and xi and xj are the abundances of species i and j. The double summations are over all pairs of species i and j (with i < j). In our study, random draws were made from different colonies in study sites as opposed to random draws from counts of individuals (for details, see sampling of ant data above).

To calculate Δ*, we generated a phylogenetic tree using genetic distances between species average mean based on the 47 sequences, using MEGA version 4 (distances in Appendix 1; Tamura et al., 2007). Since the resultant tree topology had under-estimated branch lengths at the base of the tree (Hendy & Penny, 1989; Swofford & Olsen, 1990), we used the method proposed by Grafen (1989) with the function *compute.brlen* (power = 1) to re-estimate the branch lengths of the phylogeny (Figure 1) using the package ape (Paradis et al., 2004) in R (R Core Development Team, 2009). The phylogenetic tree was then used to generate a patristic distance matrix which was used in estimating Δ*. A patristic distance is the

sum of the lengths of the branches that link two nodes in a tree, where those nodes are typically terminal nodes that represent extant species (Fourment & Gibbs, 2006), and thus a matrix of patristic distances calculated from a tree for all pairs of species summarizes the genetic change, or phylogenetic change, represented in the tree. Δ* is therefore based on a continuous measure and not on a discrete Linnaean classification (Clarke & Warwick, 2001). Use of nodes in calculating Δ* in place of patristic distances would alleviate the problem of variation in branch lengths in trees that are not ultrametric. For biological community data, species-by-site abundance matrix was used in the calculation of the diversity measure. One way ANOVA was used to assess the variation of Δ* along the succession stages.

Null model tests for phylogenetic community structure

To assess the effect of null-model choice on ability to detect processes generating community phylogenetic structure, we generated null communities using various null-models. The null model 'taxa labels' maintains both species abundances (richness) at each site, as well as the occurrence frequency of each species. It involves shuffling taxa labels across the tips of the phylogenetic tree to randomize phylogenetic relationships among species. Taxa labels were shuffled among species occurring in local communities (the local pool). Besides removing any pattern of phylogenetic clustering or over-dispersion regarding species co-occurrence within plots, randomizations with this null model also removes any pattern of phylogenetic clustering or over-dispersion regarding species abundances/frequencies (Hardy & Senterre, 2007). This model allows us to ask, given the distribution of species in communities, does the phylogenetic relatedness of species within those communities differ from random expectation?, thus allowing for testing if species within sites are more or less related than species from different sites. The model 'sample pool' maintains the total species richness of each community, with species in each community chosen equiprobably at random, without replacement from the pool of species present in the RCNR. This null model is also referred to as the ''unconstrained'' model (Kembel & Hubbell, 2006), since the species richness of each site remains the same in the null communities, but species occurrence frequencies in the null communities were not constrained to be equal to their actual occurrence frequency among sites in the RCNR

data set. This null model assumes that all species present in the RCNR are equally able to colonize any site.

Two other null models maintained both the total species richness of each site, as well as the occurrence frequency of each species. These models are referred as the ''constrained'' since the occurrence frequencies of species in the null community are constrained to be equal to their actual frequency in sites (Kembel & Hubell 2006). The models assume that a species' ability to colonize a site is proportional to its frequency in the RCNR. An example is independent swap algorithm (Gotelli, 2000; Gotelli & Entsminger, 2003) which generates constrained null communities by holding the row and column sums of the sites/species occurrence matrix constant while swapping species among sites using a checkerboard swap.

Community phylogenetic structure in each site was measured using the same phylogenetic distance matrix and community abundance data as one used in estimating Δ*. We used three indices which included: *standardized effect size* of phylogenetic diversity (sesPD), Net relatedness index (NRI) and Nearest taxon index (NTI). These measures are standardized in order to remove the effects of sample size, as opposed to phylogenetic distinctness (Δ*) index above. In particular, NRI and NTI do not account for the relative abundance of species and are not correlated with species richness. As a result, they are relatively robust to incomplete sampling (Clarke & Warwick, 1998). Both the unconstrained and constrained null models were then used to generate the null communities that we used in comparing the observed patterns, with 999 randomizations (Webb et al., 2008). Calculations of *Standardized effect size of* MPD and MNTD in communities for each site were effected using functions *ses.mpd* and *ses.mntd* respectively, implemented in R package Picante (Kembel et al., 2008). From these, NRI and NTI measures were then calculated for each sample in a manner similar to that described in Webb et al. (2002). Since the three indices gave similar community patterns, we chose to focus on the metric NTI for further analysis.

To test if the proportion of clustered communities significantly differs from the hypothesized value of 50%, we carried out a one sample binomial test for each null model. Further, to test whether the average phylogenetic structure of local ant communities at a given succession stage differed from random; we calculated the mean phylogenetic structure of all sites at each succession stage as the mean NTI of

all sites at that stage. If the mean NTI for all sites at a given succession stage differed from zero according to a one-sample t test, we could conclude that the ant communities at that stage were significantly phylogenetically clustered or over-dispersed on average. Only the null models sample pool and independent swap were used for this test. We also tested if there was correlation between species co-occurrence and phylogenetic distances using Mantel test based on 999 permutations and metrics of co-occurrence 'cij' and 'checkerboard' under the null model 'sample taxa labels' with the function *comm.phylo.cor.* in the package Picante (Kembel et al., 2008). All statistics were calculated with R version 2.10.1 (R Development Core Team 2009).

3.4 Results

Our analysis was based on determining phylogenetic composition using index Δ* and standardized indices (sesPD, NRI & NTI), and further using the latter three indices in determining the phylogenetic community patterns under various null models. Since the three standardized indices gave similar patterns, we focus only on NTI measure (see Appendix 2, for phylogenetic spread of species). Δ* showed an increasing trend along the forest succession stages. Furthermore, it was positively and significantly correlated to species richness (Figure. 2 A and B respectively) and significantly varied between succession stages ($F_{3,\ 8} = 7.82$, $P = 0.01$). Patristic distances generated using phylogeny whose branch lengths were modified using the method 'grafen', were highly correlated to original distances ($r = 1$, $p = 2.2e{-}16$). NTI measure (Table 1) revealed no correlation between both Δ* and species richness ($r = 0.1$, $P > 0.1$; $r = 0.2$, $P > 0.1$ respectively, based on null models sample pool and independent swap). Phylogenetic community patterns did not significantly vary between succession stages ($F_{3,\ 8} = 0.01$, $P > 0.1$; for sample pool and independent swap null models).

Based on the unconstrained null models (taxa labels, richness and sample pool), all 11 sites had positive NTI values (phylogenetically clustered ant communities). Sites H2, M1, M3, F1 and F2 showed some significant clustering ($P < 0.1$); while site F3 tended to have a close to random community pattern under these three models (Table 1). The constrained (independent swap and trial swap) null models showed similar community patterns (Table 1), which is attributable to their

shared tendency of ‘‘Narcissus effect’’ (see discussion). NTI values under the null model frequency were negative for sites H1, A1, A3, M2 and F3, with the remaining six sites having positive values. Sites A1 and M2 tend to have almost random communities using this null model i.e. NTI values are near zero (Table 1). Binomial tests revealed that the proportion of clustered communities significantly differ from the hypothesized value of 50% (P = 0.001, for the null models taxa labels, richness and sample pool); no statistical significant difference (P = 0.227, for null models trial swap and independent swap) and P = 1.00 for the null model frequency. Mean NTI values generated using null models sample pool and independent swap showed that only ant communities in young secondary forest (stage A) were significantly different from zero (t = 67.68, P =0.00; t =5.29, P = 0.03 for the two models respectively). Correlation test between species co-occurrence and phylogenetic distances revealed no correlation (r = 0.05, P = 0.34 for Mantel test; and r = 0.03, P = 0.7; r = 0.04, P = 0.6 for cij and checkerboard metrics respectively).

Table 1. NTI values for 11 sites in Rio Cachoeira Nature Reserve calculated using unconstrained and constrained null models. Asterisk (*) indicates significant levels at 0.1. Site H3 was removed since it had only 1 taxa.

sites	ntaxa	taxa labels	Richness	Sample pool	Trial swap	Independ-ent swap	Frequency
H1	5	0.76	0.77	0.75	-0.06	-0.04	-0.50
H2	3	1.49*	1.52*	1.57*	0.89	0.65	0.09
A1	6	0.99	0.99	0.96	0.19	0.27	-0.03
A2	9	0.99	1.00	0.98	0.78	0.32	0.50
A3	6	0.98	0.99	1.01	0.40	0.16	-0.15
M1	9	1.22*	1.24*	1.25*	0.89	1.19*	0.99*
M2	11	0.28	0.23	0.25	-0.68	-0.99	-0.02
M3	11	1.33*	1.34*	1.34*	0.69	1.06	0.86
F1	10	1.40*	1.41*	1.39*	0.95*	1.11*	0.92*
F2	11	1.07*	1.05*	1.08*	0.76	0.87	0.84
F3	10	0.08	0.02	0.04	-1.66	-1.70	-0.34

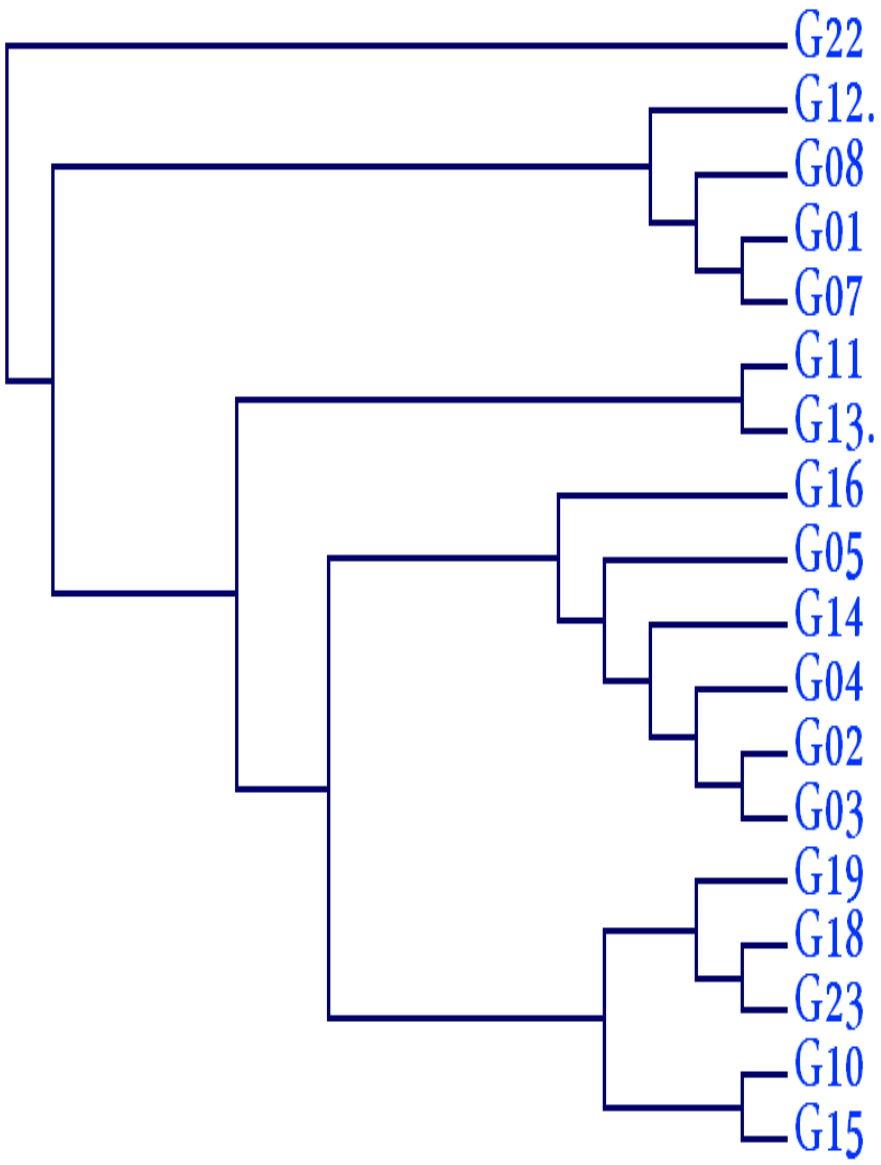

Figure 1. A phylogeny of 18 species occurring along the forest succession stages in Rio Cachoeira Nature Reserve. The species included are based on identification criterion in chapter 2 where each taxa reprtesents a MOTU. The branch lengths of the phylogeny are adjusted using the method Grafen (Grafen, 1989).

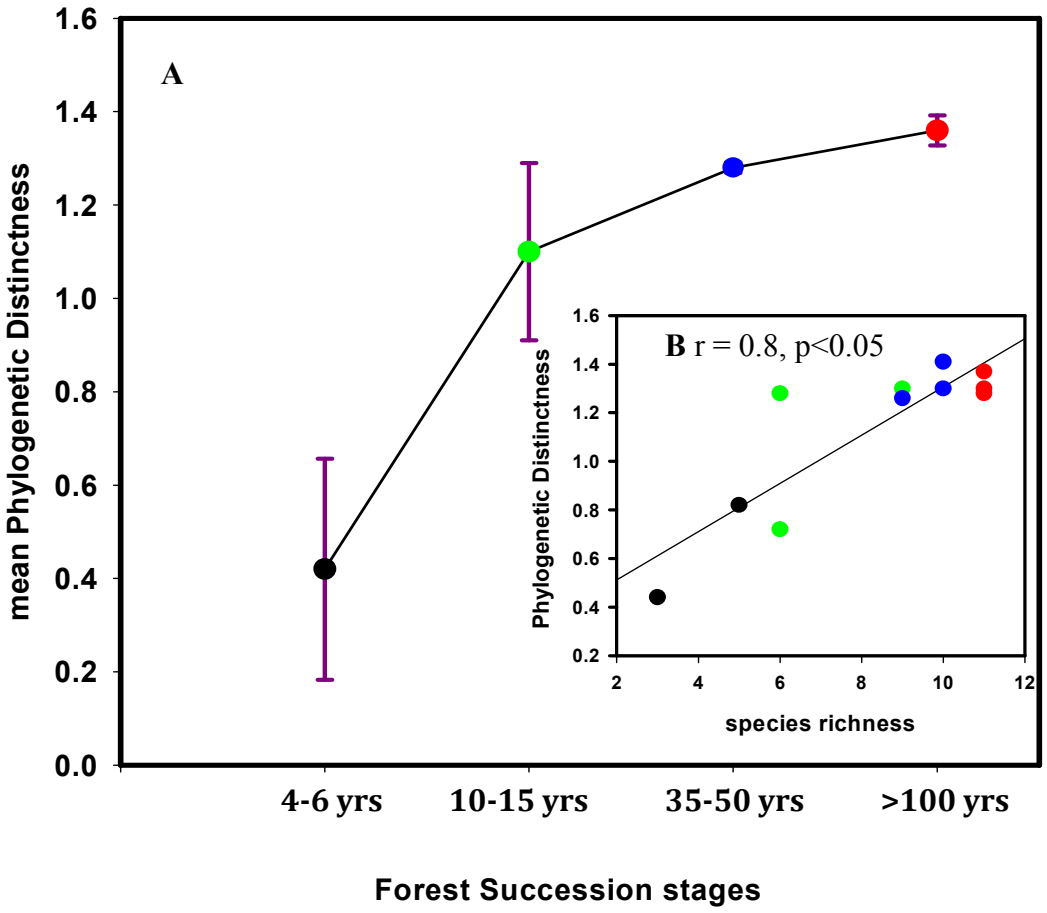

Figure 2. The trend of mean Phylogenetic distinctness ± SE along forest succession stages in Rio Cachoeira Nature Reserve (A) and Phylogenetic distinctness plotted against species richness (B). Phylogenetic distinctness tends to increase with increase in species richness. Each colour for the symbols in Figure 2B represents different succession stages.

3.5 Discussion

Our findings showed that phylogenetic composition of *Pheidole* ants is sensitive to forest succession, just as is species richness. This implies that the recovery of the secondary forests is accompanied by an increase in phylogenetic distinctness and species richness in this genus. Increase in species richness along the gradient of secondary tropical forests has also been observed for ant genera in the study area (Bihn et al., 2008). These increases may be attributed to the suitability of the local environment for species adapted to it, meaning that the number of species in each succession stage is then limited by the number of species in the regional species pool adapted to the local environment (Bihn et al., in prep). Studies have noted that leaf litter ants are known to react sensitively to variations in the microclimate of the leaf litter (Whitford & Ettershank, 1975; Kaspari, 1993). In that case, low phylogenetic distinctness of *Pheidole* ants at early forest succession stages may be caused by extremes in the microclimate (high temperature and low leaf litter) and these might have excluded ant species in this genus which are adapted to a narrow range of temperature and humidity typical of older forest stages. This is likely so because nest availability, food abundance and microclimatic stability are all likely mediated by the depth of the leaf litter (Bihn et al., in prep).

Our analyses further showed that *Pheidole* ant communities had same values of Δ^* at various sites (results not shown) i.e. same patristic distances but there species composition can be different. This may in part reflect the behaviour of Δ^* and other relatedness measures based on average properties, to 'saturate' (reach maximal values with increasing sample size; see Warwick & Clarke, 1995; Izsak & Price, 2001). Furthermore, Δ^* being a non-parametric test depends on the system studied and is not comparable with values obtained from different studies. Nevertheless, it is possible to compare the relative placement of sites/areas in different rankings (Posadas et al., 2000).

On overall both the unconstrained and the constrained null models revealed phylogenetically clustered communities, indicating that *Pheidole* species occurring together in each site are more closely phylogenetically related than expected by chance (Webb, 2000; Kembel & Hubbell, 2006). This may imply that habitat

conditions (e.g. availability of food, nests) influence the observed patterns as opposed to the effects of the null models used, especially for the unconstrained models which showed clustered ant communities for all the 11 sites. Clustering of ant communities indicates that habitat-use is a conserved trait within the pool of species in the community; interpreted as evidence of habitat selection for ecologically similar, phylogenetically related species (Webb, 2000; Webb et al., 2002; Cavender- Bares et al., 2004). The observed over-dispersion in certain sites using constrained null models is likely to be the effect of null models (the ''Narcissus effect'' [Colwell & Winkler 1984]), as opposed to effects of succession. Bihn et al. (in prep) notes that, due to spatial heterogeneity of the environment, the effects of interspecific competition leading to over-dispersion may fail to be reflected in ant communities in this genus. The almost random communities in some sites could be an indication that the opposing forces of habitat filtering and interspecific competition more or less counteract one another (Webb et al., 2002; Kraft et al., 2007).

The idea that *Pheidole* ant communities in our study likely form non-interactive assembly contrasts with many studies which suggest that competitive interactions structure ant communities (Levings & Traniello, 1981; Savolainen & Vepsäläinen, 1988; Morrison, 1996; Hölldobler & Wilson, 1990). This is however not surprising since many ant species, with some in this genus included, are opportunistic scavengers feeding on a variety of food items like dead insects, fruit fragments, seeds and many others (Carrol & Janzen, 1973; Coelho & Ribeiro, 2006). The availability of these food items in the leaf litter are highly unpredictable in space and time which make them difficult to monopolize and exclude other species from this resource. This study concurs with other research findings that resource availability and habitat conditions are the main driving forces structuring communities of tropical leaf litter ants (Byrne, 1994; Kaspari, 1996a; Kaspari, 1996b; Soares & Schoereder, 2001; Theunis et al., 2005; Bihn et al., in prep).

The suitability of a given null model in the study of community structure is still being debated (Gotelli, 2001). While models that do not maintain species frequencies have been criticized as being overly statistically liberal; a shortcoming described earlier as the ''Jack Horner effect'' (Wilson, 1995), null models that maintain species frequencies have been criticized for potentially ''smuggling in'' the effects of processes such as competition or environmental filtering on species frequencies and

community phylogenetic structure (the ‘‘Narcissus effect’’ [Colwell & Winkler 1984]), and for potentially being too statistically conservative, as is the case with independent swap algorithm. Regardless of these criticisms, on overall both unconstrained and constrained null models in this study gave consistent results i.e. clustered phylogenetic communities.

Implications to conservation

On overall, our results suggest higher conservation priority for old-growth forests with the more phylogenetically distinct taxa. This concurs with other studies that by conserving the remnants of the old growth forests in Brazilian Atlantic forest, most of the biodiversity would be protected as well (Bihn et al., 2008, 2010). Conservation decisions on areas of priority can also be based on choosing the most phylogenetically diverse and significantly clustered site combinations. Phylogenetic clustering within a locality presents an important scenario for biodiversity conservation because loss of such a locality would mean loss also of the deeper phylogenetic branches linking its member taxa. Conservation of site combinations as opposed to a single succession stage would cater for rare species only traced at specific sites. Rare species largely contribute to diversity, and also can make significant contribution to ecosystem functioning (Lyons et al., 2005), and their loss would mean loss both from an ecological and an evolutionary perspective. Biodiversity conservation strategies should thus adopt a form of risk analysis that involves estimating pattern of diversity variation, and then trying to conserve as much of that estimated variation as possible for the future (Faith & Baker, 2006).

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Chapter 4

4 Determinants of phylogenetic community structure of ant genera along succession gradient in secondary tropical forests

4.1 Abstract

The mechanisms leading to phylogenetic community structure in local assemblages have become a major focus in recent community studies. Studying the phylogenetic structure of an ecological community can provide insights into the relative importance of different processes structuring that community. This study aimed at measuring the phylogenetic structure of ant genera communities occurring in 12 sites along a forest succession gradient, in the tropical secondary forests of Brazil. We also determined the processes structuring the ant communities; and tested the influence of metrics used, succession and taxonomic scale on the estimates of phylogenetic community structure. On average, the phylogenetic structure of ant communities was over-dispersed, meaning that ant species were more distantly related to their neighbours than expected by chance. The observed over-dispersion and clustering across the sites depended on the metric used and to an extent the forest succession stage, although other possible explanations may include effects of the null model, variation in the strength of ecological processes among habitats or distribution of traits. We noticed that finely defined ant communities (single genus) tended to be clustered while with several ant genera, most communities showed over-dispersion, suggesting that competition is increasingly evident as ant communities are defined to include greater phylogenetic diversity. This study shows that the phylogenetic structure of ant communities depends on interplay of several factors, most of which still need to be comprehensively researched on.

Key words: Overdispersion, phylogenetic clustering, succession, taxonomic scale

4.2 Introduction

The phylogenetic structure of ecological communities has become a major focus of recent research (Webb, 2000; Webb et al., 2002; Cavender-Bares et al., 2004, 2006; Kembel & Hubbell, 2006). However, most studies especially in species rich tropical ecosystems have largely focused on plant communities (Chazdon et al., 2003; Kembel & Hubbell, 2006; Letcher, 2010). Much attention is yet to be given to invertebrates such as ants, despite their abundance, species richness (Floren & Linsenmair, 2000) and ecological dominance (Fittkau & Klinge, 1973; Rockwood & Glander, 1979; Floren et al., 2002; Rico-Gray & Oliveira, 2007).

Most tropical forest ecosystems are recovering from anthropogenic impacts and are at different stages of succession (Osorio-pérez et al., 2007; Bihn et al., 2008a & b, 2010). Ecological succession consists of a series of changes in time affecting both biocenotic composition and an ecosystem's physical environment (Zorilla et al., 1986). In ecosystems undergoing succession such as secondary tropical forests, ants (especially those dwelling in leaf litter) are important due to their major role in ecosystem processes especially in facilitating decomposition of leaf litter (Chung, 1995; Shik & Kaspari, 2010), seed dispersal and predation (Torres & Medina-Gaud, 1998), and can therefore influence the pattern of forest recovery (Didham et al., 1996). Due to their social organization and soil nesting habits, ants are highly dependent on many environmental factors (Wilson, 1971), and structural conditions created by the vegetation in terms of availability of habitats and resources (Zorilla et al., 1986). It is therefore probable that several ant genera would show changes in their spatial organization during succession related to the different degree of "maturity" shown spatially and temporally by the pasture /vegetation (see Austin, 1977; Pineda et al., 1981). We also expect that competition (McGlynn & Kirksey, 2000), and or habitat effects (Torres, 1984; Perfecto & Vandermeer, 1996; Carvalho & Vasconcelos, 1999; Kaspari & Weiser, 2000; Oliver et al., 2000) may play a key role in determining how these ants are assembled along the succession stages. These two processes i.e. competition and habitat filtering are often considered as central to the assembly of communities and lead to opposite predictions about the phenotypic

similarity and phylogenetic relatedness of co-occurring species (Tofts & Silvertown, 2000; Webb et al., 2002).

Ecological patterns and processes are scale-dependent, with observations at one scale often not applying to other scales (Wiens, 1989; Levin, 1992; Schneider, 1994). For instance, community assembly patterns have shown dependency on how one delineates the taxonomic scale of the local assemblage and the reference species pool (Cavender-Bares et al. 2006). However, the few studies that have used community phylogenetics in the context of scale have provided mixed results concerning whether communities are phylogenetically clustered (Webb, 2000), phylogenetically overdispersed (Cavender-Bares et al., 2004), or show no phylogenetic structure (Kembel & Hubbell, 2006). Recent evidence from Cavender-Bares et al. (2006) and N. G. Swenson, B. J. Enquist, J. Thompson, and J. K. Zimmerman (unpublished data) shows that the above studies are all prone to scale dependency. Swenson et al. (2006) suggests that, as the local assemblage becomes more finely defined spatially, phylogentic over-dispersion is common. However, this argument contrasts with our observation in a previous study on ant communities involving a single genus which showed phylogenetic clustering for all study sites (see chapter 3). We therefore expect that, with the inclusion of several ant genera in a community species pool, the conclusions on resultant phylogenetic structure may either be random, clustered or over-dispersed (Webb, 2000), and this can further be influenced by aspects such as metrics or null model used in testing community pattern.

In this study, we examine how leaf litter ant genera are phylogenetically structured along the forest succession stages in the tropical secondary forests of Brazil, and the processes/determinants leading to the observed assembly patterns. We also aim at comparing community patterns observed when using a single ant genus (see chapter 3) and those resulting when several ant genera are in consideration. In achieving this, our study focused on answering the following research questions: (1) How are ant communities structured along the forest succession gradient? (2) What are the processes structuring ant communities along the forest succession gradient? (3) Does succession influence phylogenetic structure of ant communities? (4) How does taxonomic scale influence phylogenetic community structure of ants along a successional gradient?

4.3 Materials and Methods

Community data and genus level phylogeny

Community sample data was obtained from leaf litter ants sampled from 12 study sites scattered across the Rio Cachoeira Nature Reserve (RCNR). Sampling was done between June and September 2003. The sites were organized as per the four stages of secondary forest succession, whose ages were: very young secondary forest (4-6 years), young secondary forest (10-15 years), old secondary forest (35-50 years), and old-growth forest (> 100 years; Bihn et al., 2008b, 2010). There were three site replicates for each succession stage and the replicated sites of a particular succession stage were separated by a distance of 4 km (range=1-6 km). For details on sampling methodology and identification of specimens into morphospecies see Bihn et al. 2008b, 2010).

Indices for measuring phylogenetic community Structure

Because adding species into a sample alters the topology of the phylogenetic network joining them, most 'raw' metrics are correlated with the taxon richness of the sample (Vamosi et al., 2009). Various standardized metrics have thus been developed to enable comparisons of phylogenetic structure among samples of different species richness i.e. 'standardized effect size' (Gotelli & Rohde, 2002; Kembel & Hubbell, 2006). Three measures dealt with in this section include: the standardized effect size phylogenetic diversity (sesPD) as used by Proches et al. (2006), Net relatedness index (NRI) and Nearest Taxon Index (NTI), obtained in a manner similar to that described in Webb et al. (2002).

Measurement of community phylogenetic structure

To investigate the phylogenetic structure of ant communities at each site, we used the genus level phylogeny proposed by Brady et al. (2006) which we further modified by pruning and also incorporating certain taxa. This was achieved by pruning all genera in Brady's phylogeny that were absent in our abundance data matrix using the function *drop.tip*, and replacing them with genera occurring in the abundance data but absent in phylogeny to form a polytomy (see Appendix 3). In order to correct for any under-estimation of branch length in the modified phylogeny (Hendy & Penny, 1989; Swofford & Olsen, 1990), we used the method proposed by Grafen (1989) with the function *compute.brlen* (power = 1) to re-estimate the branch lengths of the phylogeny (Figure 1). For comparison purposes, we also generated branch lengths by setting all branch lengths to 1. This approach is useful in comparative analyses when a phylogeny with no branch lengths is available. Patristic distances resulting from the two phylogenies were correlated to establish the relationship. In most studies, the method grafen is more preferred in adjusting branch lengths of phylogenies because it offers a standard way of generating an ultrametric tree (Mathews et al., 2011). An ultrametric tree is a rooted additive tree where the terminal nodes are all equally distant from the root. All functions for phylogeny adjustments were implemented using the package ape (Paradis et al., 2004) in R (R Core Development Team, 2009).

Phylogenetic structure of the ant community was estimated using the three 'Standardized effect size' indices earlier described. Null communities were generated using Independent swap algorithm developed by Gotelli & Entsminger (2003). In this null model, the row and column sums of the original matrix are preserved. Thus, each random community contains the same number of species as the original community (fixed column total), and each species occurs in the same frequency as in the original community (fixed row total; Connor & Simberloff, 1979). This algorithm has good statistical properties (low frequency of Type I and Type II errors) when tested against random and structured matrices (Gotelli, 2000; Gotelli & Entsminger, 2001). We reshuffle the data in a species *x* sample presence/absence matrix (see chapter 3 on null model tests; Webb et al., 2008 for details). Thus, this approach does not rely on the entire phylogeny to define the possible species pool from which species are

drawn. Instead, it uses only the taxa observed in the study for which phylogenetic structure is being estimated.

The Standardized effect size of phylogenetic diversity (*ses*PD) was calculated using community abundance data, the genus level phylogeny (Figure 1), using null model independent swap. Calculation was effected using function *ses.pd* in the package Picante (Kembel et al., 2008) in R. For the two other measures, the standardized effect measures of MPD and MNND/MNTD were first calculated using functions *ses.mpd* and *ses.mntd* respectively, from which NRI and NTI were calculated using two equations as described by Webb et al. (2002).

To test whether the average phylogenetic structure of local ant communities at a given succession stage differed from random; we calculated the mean phylogenetic structure of all sites at each succession stage as the mean NRI and NTI of all sites at that stage. If the mean NRI or NTI for all sites at a given succession stage differed from zero according to a one-sample t test, we could conclude that the ant communities at that stage were significantly phylogenetically clustered or overdispersed on average, since both NRI and NTI are standardized effect sizes whose expected values are zero for phylogenetically random communities, positive for phylogenetically clustered communities, and negative for phylogenetically over-dispersed communities (Gotelli & Rohde, 2002). All analyses were carried out in R (R Development Core Team, 2009).

For taxonomic scaling, we used the observed community patterns using a single genus *Pheidole* (see chapter 3) and compared the results with the community patterns observed when several ant genera are included in the species pool i.e. in this study.

4.4 Results

Our results on the branch length re-estimation of phylogeny showed that grafen method leads to an ultrametric tree as opposed to setting all branch lengths to 1, and the patristic distances from the two phylogenies were relatively correlated ($r = 0.73$, $P = 2.2e\text{-}16$). Our further results were based on patristic distances from the phylogeny with branch lengths adjusted using method 'grafen'. The three measures were not correlated with genera richness, and showed a hump-like pattern along the

forest succession stages (Fig 2). sesPD was negatively and moderately correlated to NRI (r = – 0.5; P = 0.1) and NTI (r = – 0.61; P = 0.04). The NRI and NTI means were not significantly different from zero (P > 0.05). The overall means for sesPD, NRI and NTI for the 12 sites were – 0.02 ± 0.3, 0.06 ± 0.3 and –0.33 ± 0.3 S.E respectively. Phylogenetic community patterns did not significantly vary between succession stages ($F_{3, 8}$ = 0.38, P > 0.5; for sesPD and NRI metrics) and ($F_{3, 8}$ = 2.16, P > 0.1 for NTI).

Furthermore, the phylogenetic community structure patterns varied along the forest succession stages depending on the metrics. Five sites had negative sesPD (phylogenetically clustered) while seven had positive sesPD (phylogenetically over-dispersed). Ant community in site A2 showed some level of significant clustering (P = 0.1; Table 1). According to NRI index, six communities had negative values (phylogenetically over-dispersed) while six others had positive values (phylogenetically clustered). Ant community in site H3 was significantly clustered (P = 0.05) and that in A1 showed an almost random community structure with its NRI value of 0.05 (Table 1). Nine communities had negative NTI values (phylogenetically over-dispersed) and only three communities showed positive values (phylogenetic clustering). Communities in sites A2 and A3 were significantly clustered (P = 0.05), while site F3 showed an almost random community with NTI value of – 0.02 (Table 1). Our taxonomic scaling analyses showed that as the taxonomic scale became broader, the level of phylogenetic over-dispersion increased (Figure 3).

Table 1. Species richness (SR), Standardized effect size PD (sesPD), NRI and NTI measures for the genus level phylogeny for 12 sites in Rio Cachoeira Nature Reserve using the null model Independent swap. Significance levels: **= 0.05; *= 0.1.

Sites	SR	sesPD	NRI	NTI
H1	13	1.48	-1.00	-0.56
H2	14	0.82	0.76	-0.45
H3	12	-1.65	1.94**	-0.21
A1	15	0.33	0.05	-0.78
A2	16	-1.39*	-0.84	1.27**
A3	20	-1.01	-0.48	1.36**
M1	21	1.60	0.79	-0.88
M2	24	-0.80	1.13	-0.89
M3	22	0.23	-0.92	0.16
F1	27	0.99	-1.25	-1.60
F2	26	0.65	-1.38	-1.40
F3	28	-1.51	1.88	-0.02

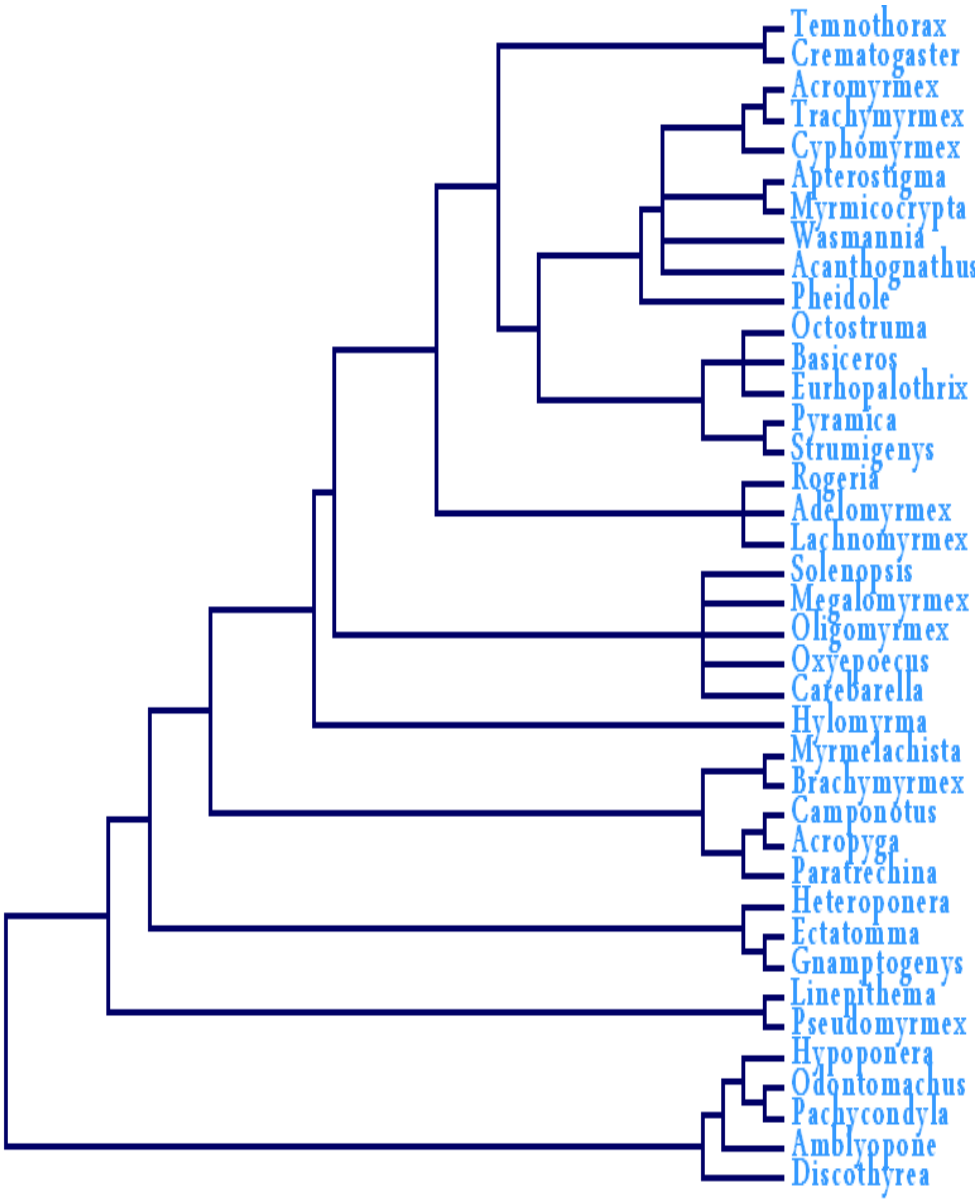

Figure 1. A genus level phylogeny of taxa occurring in Rio Cachoeira Nature Reserve. Phylogeny modified from that of Brandy et al. (2006), and the branch lengths adjusted using method 'grafen' as proposed by Grafen (1989).

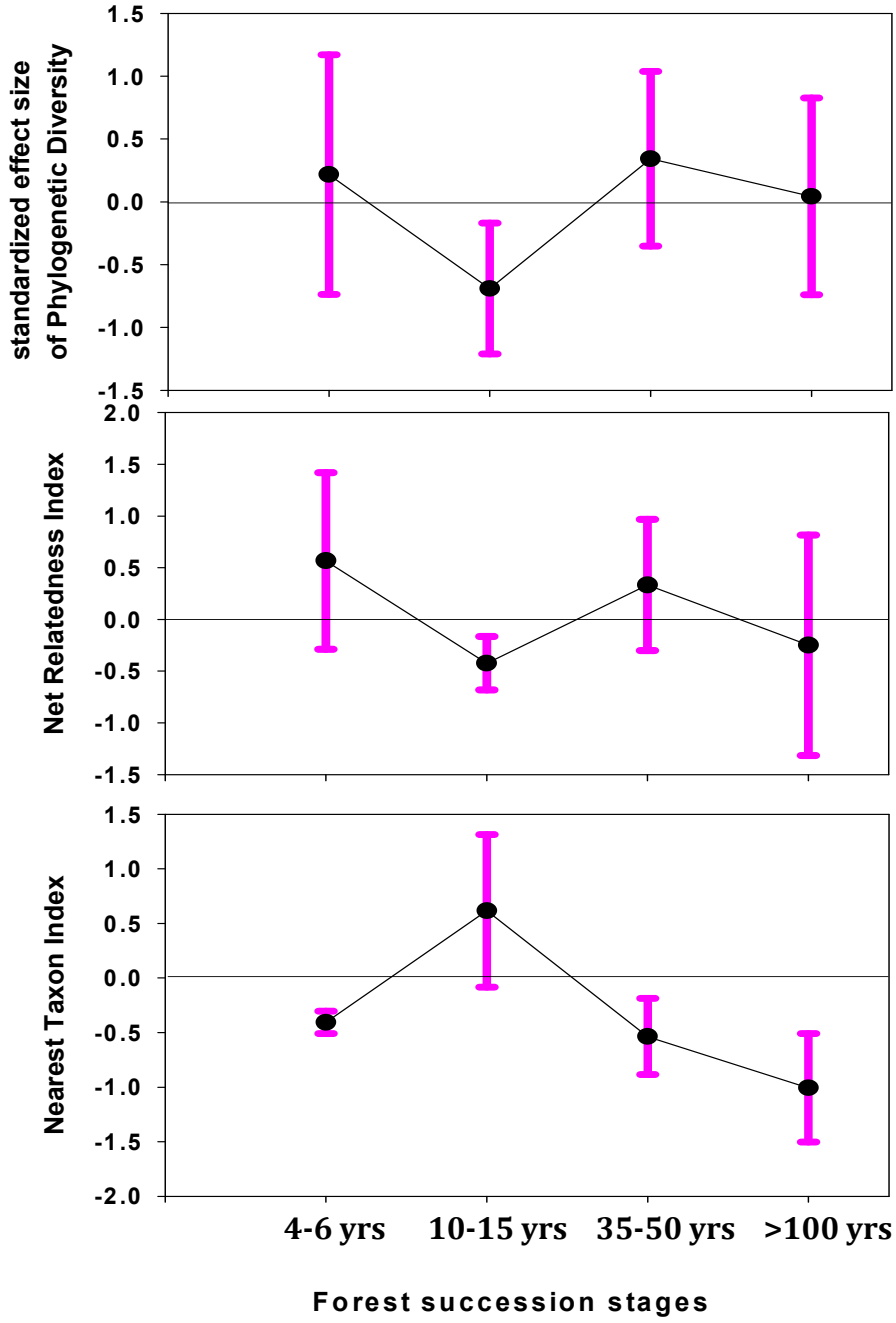

Figure 2. Error bars (mean ± 1 S.E) showing the average phylogenetic community structure of ant genera, using standardized effect size of Phylogenetic Diversity (sesPD), Net relatedness index (NRI) and Nearest taxon index (NTI). Points above and below zero line indicate phylogenetically over-dispersed and clustered communities respectively for sesPD. The interpretation of NRI and NTI values is the opposite of this.

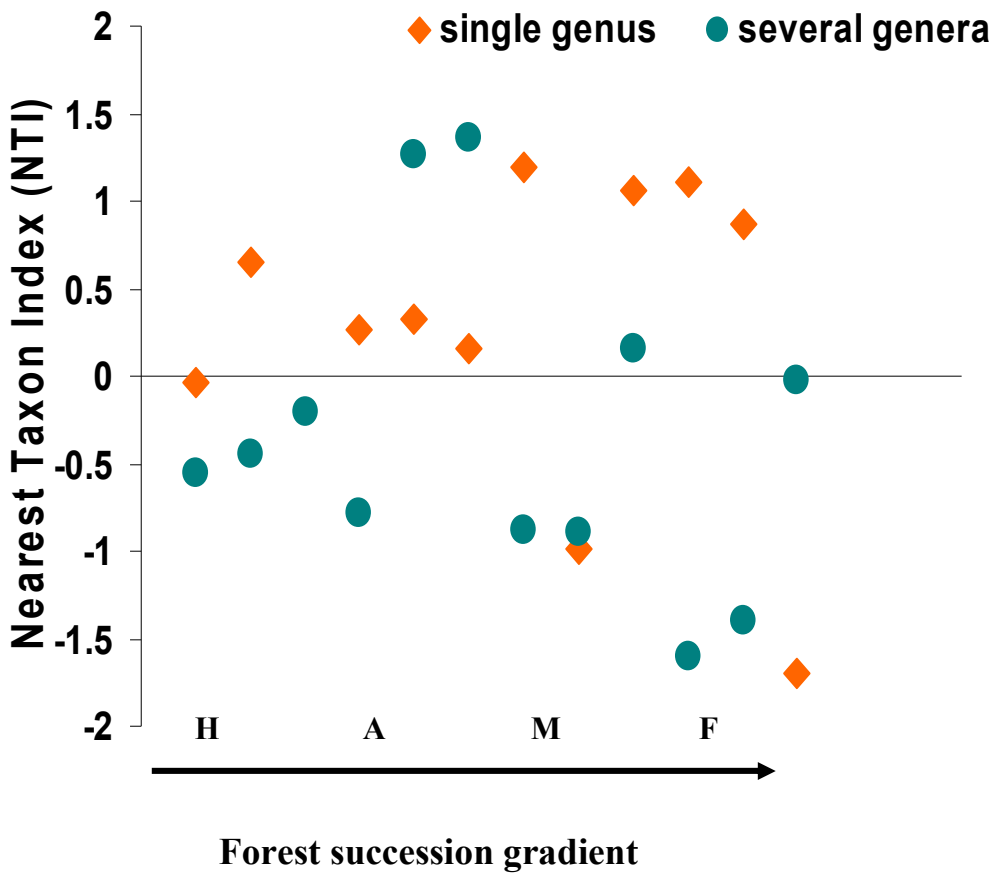

Figure 3. Distribution of ant communities at two taxonomic scales i.e. single genus (*Pheidole*) and several ant genera. Most ant communities involving several ant genera tend to be highly phylogenetically over-dispersed in older succession stages, and for the single genus, clustering was more evident. Nearest Taxonomic Index (NTI) measures for the two taxonomic scales are based on Independent swap algorithm. Sites **H** represent youngest secondary forest (4-6 years), **A** young secondary forest (10-15 years), **M** old secondary forest (35-50 years) and **F** old-growth forest (> 100 years). Each site has three replicates.

4.5 Discussion

Our results show that patterns of co-occurrence can deviate from random expectations with regard to phylogenetic relatedness of species in different genera, resulting to either phylogenetic over-dispersion or phylogenetic clustering. These patterns are seen to vary depending on the metric used, which may be attributed to sensitivity of metrics to different aspects of community structure (Webb, 2000; Vamosi et al., 2009). We may also not rule out the influence of the used null model on the observed community patterns. Independent swap algorithm which maintains species frequencies, has been criticized for potentially ‘‘smuggling in’’ the effects of processes such as competition or environmental filtering on species frequencies and community phylogenetic structure (the ‘‘Narcissus effect’’ [Colwell & Winkler 1984]).

On average ant communities in RCNR are phylogenetically over-dispersed regardless of the metric, suggesting that competitive exclusion limits the coexistence of closely related species in the communities, because they compete for the same limiting resources (Webb et al., 2002; Cavender-Bares et al., 2006). The varying patterns i.e. hump-like pattern along the forest succession stages (Figure 2) may be an indicator of varying abiotic conditions, and that resources for most genera are not evenly distributed along the forest gradient. Several environmental conditions, including soil moisture, depth to water table and temperature at the ground surface (factors not addressed in this study), are likely to vary along the forest succession gradient, both as cause and/or as effect (Andersen, 1995). Even seemingly minor changes in these conditions can be important to individual ants/or genera. Succession stages rich in particular resources or favourable abiotic conditions may be more preferred by some litter ant genera to others, and community patterns may depend on whether these resources are distributed in a homogeneous or heterogeneous way in various sites. According to Bihn et al. (2008a), some leaf-litter dwelling genera are restricted to old-growth forests, suggesting that critical resources on which such genera rely on are distributed in a similar nested way.

Studies have observed that competitive interactions for ant communities might be particularly important in more homogeneous habitats (Chong et al., 2010), since homogeneity offers a lower variety of opportunities with respect to resources and

microclimatic conditions (Kaspari, 1996; Campos *et al*., 2003). On the other hand, heterogeneous habitats are likely to allow species coexistence due to greater availability and better quality of nesting sites, food, and favourable abiotic and microclimatic conditions (Pacheco et al., 2009; Chong et al., 2010). This may also explain the over-dispersion or clustering of ant communities at various sites along the succession gradient.

Further, our results suggest that there seem to be a pattern of scale dependency resulting from the taxonomic delineation of local assemblages where more finely taxonomically defined communities (single ant genus) are phylogenetically clustered (see chapter 3), while with inclusiveness of taxa e.g. several ant genera as in this study, most communities show phylogenetic over-dispersion. These findings suggests that environmental filtering is more dominant process than competitive exclusion in determining community membership for ant species in that single genus as compared to several genera along the forest succession gradient. This allows a reconciliation of apparently contradictory results, as both niche conservatism and species interactions are important forces in community assembly, but are dominant at different scales (see Cavender-Bares et al., 2006). Research findings from studies by Cavender-Bares et al. (2006) and Swenson et al. (2006) differ with how ant communities are structured at different taxonomic scales along the succession gradient as per this study, implying that observed phylogenetic structure patterns may depend on multiplicity of factors such as the spatial scale in consideration or the phylogenetic distribution of important traits (Webb et al., 2002).

Conclusions

In this study we have demonstrated how several aspects influence the phylogenetic structure of ant communities. The observed assembly patterns suggest that the different forest succession stages provide different combinations of resources and microhabitats for the ant genera. We notice that with the inclusiveness of more taxa (several ant genera) in the species spool, the ants communities tend to be phylogenetically over-dispersed, an observation contrasting other studies (e.g. Cavender-Bares et al., 2006; Swenson et al., 2006). This suggests that multiple factors are likely to determine community structure and therefore need to be studied more comprehensively. For instance, examining the conservatism in ecologically relevant

functional traits in relation to their distributions across successional/environmental gradients can help decipher the processes that cause phylogenetic structure in communities (Cavender-Bares & Wilczek, 2003). Moreover, the spatial scales at which the community is being studied need be taken into account, since this also determines resource distribution and suitable microhabitats (Wiens, 1989; Menge & Olson, 2003). The local habitat characteristics and regional environmental factors need to be tested during phylogenetic community studies since these mediate biotic interactions and are important in interpreting processes that lead to community assembly (Helmus et al., 2007).

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Summary

5 Summary

Habitat disturbance leading to succession especially in species rich tropical forests is currently a main challenge for community ecologists and conservation biologists. This is because it results to biodiversity losses, thus affecting species composition and altering the ecological processes that structure communities. The first step to effective conservation planning is the identification of the occurring biodiversity, assessing the phylogenetic composition and phylogenetic structure especially for ecologically hyper-diverse invertebrate communities such as ants in such threatened habitats. In this regard, we assessed the diversity of a hyperdiverse ant genus *Pheidole* in Rio Cachoeira Nature Reserve within the Atlantic forest of Brazil. Using both DNA-barcodes (mtDNA cytochrome oxidase 1 gene) and morphological taxonomy, we identified 19 MOTUs and 20 morphospecies respectively for the two methods. Although the two species identification methods did not give same diversity estimate due to occurrence of distinct mitochondrial lineages within morphological species, they still can offer an effective tool for biodiversity inventories.

On examining the phylogenetic composition and community structure of ants in this genus, we found that local communities are composed of closely related ant assemblages. The old growth forests were on overall the most phylogenetically diverse habitats, thus warranting high conservation priority. Regardless of the null models used, ant communities in the genus *Pheidole* were phylogenetically clustered, implying that environmental filtering plays a major role in structuring these communities, and that habitat use is a conserved trait. Phylogenetic community patterns were not influenced by succession stages regardless of an increase in species richness along the forest succession gradient, which is attributed to habitat heterogeneity in terms of resource availability and abiotic conditions.

An exploration on how various ant genera are structured in Rio Cachoeira Nature Reserve revealed that on overall most communities were phylogenetically over-dispersed, meaning that competitive interactions is important in structuring these communities. This shows that at different taxonomic scales, processes structuring communities are likely to differ and so will the expected assembly patterns.

In conclusion, we propose that future systematics on *Pheidole* ants should use a combination of methods in overcoming identification problems such as distinct mitochondrial lineages within morphological species. As far as ecological community studies on phylogenetic composition and structure are concerned, measuring of the traits for the organisms and also the environmental variables in the habitat would allow for better interpretation of ecological processes structuring ant communities and the resultant patterns. Finally, conservation efforts need be largely concentrated in the phylogenetically diverse old growth forest remnants to ensure maximum biodiversity is preserved.

6 Zusammenfassung

Die Zerstörung von Lebensraum, welche besonders in artenreichen tropischen Wäldern zu Sukzession führt, ist gegenwärtig eine der großen Herausforderungen für Ökologie und Naturschutz. Sie führt zum Verlust von Biodiversität und beeinflusst dadurch die Artzusammensetzung ebenso wie die ökologische Prozesse, welche Artengemeinschaften strukturieren. Der erste Schritt zu einer effektiven Naturschutzplanung ist die Identifizierung der vorhandenen Biodiversität und die Bewertung der phylogenetischen Zusammensetzung und Struktur, besonders für ökologisch hoch diverse Gemeinschaften von Invertebraten wie z.B. Ameisen in solch bedrohten Habitaten. In diesem Kontext untersuchten wir die Diversität der hoch diversen Ameisengattung *Pheidole* im "Rio Cachoeira Nature Reserve" im atlantischen Wald von Brasilien. Mit Hilfe von DNS-Barcodes (mtDNS Cytochrome Oxidase 1) und morphologischer Taxonomie identifizierten wir 19 MOTUs bzw. 20 Morphospezies. Obwohl die beiden Methoden zur Artbestimmung nicht identische Schätzungen aufgrund des Auftretens von verschiedenen mitochondrialen Linien innerhalb der morphologischen Arten ergaben, bieten sie dennoch ein effektives Werkzeug für die Inventarisierung von Biodiversität. Durch die Untersuchung der phylogenetischen Zusammensetzung und Struktur der Artengemeinschaft von Ameisen dieser Gattung fanden wir heraus, dass lokale Gemeinschaften aus nah verwandten Ameisengruppen zusammengesetzt sind. Die Urwälder stellten insgesamt die phylogenetisch diversesten Habitate dar und berechtigen dadurch eine hohe Priorität im Naturschutz. Unabhängig von den verwendeten Nullmodellen waren die Ameisengemeinschaften der Gattung *Pheidole* phylogenetisch gebündelt, was impliziert, dass Umweltfilter eine wichtige Rolle in der Strukturierung dieser Gemeinschaften spielen und dass die Habitatnutzung eine konservative Eigenschaft ist. Phylogenetische Muster der Gemeinschaft waren nicht beeinflusst von Sukzessionsstadien, unabhängig von einem Anstieg des Artenreichtums entlang des Waldsukzessionsgradienten, welcher auf Habitatheterogenität im Hinblick auf Ressourcenverfügbarkeit und abiotische Bedingungen zurück geführt wird. Eine Untersuchung über den Strukturreichtum von Ameisengattungen im "Rio Cachoeira

Nature Reserve" enthüllte, dass die Gemeinschaften im Allgemeinen phylogenetisch überverteilt waren, was bedeutet, dass Konkurrenz eine wichtige Rolle in der Strukturierung dieser Gemeinschaften spielt. Dies zeigt, dass sich die Prozesse, welche Artengemeinschaften strukturieren, auf unterschiedlichen taxonomischen Skalen wahrscheinlich unterscheiden und folglich auch die erwarteten allgemeinen Muster der Biozönose. Folglich schlagen wir vor, dass zukünftige Arbeiten an der Systematik von Ameisen der Gattung *Pheidole* eine Kombination von Methoden verwenden sollten, um Identifikationsprobleme wie verschiedene mitochondriale Linien innerhalb morphologischer Arten zu überwinden. Bei ökologischen Studien über die phylogenetische Zusammensetzung und Struktur von Gemeinschaften würden Messungen der Eigenschaften der Arten und von Umweltvariablen im Habitat bessere Interpretationen der ökologischen Prozesse welche Ameisengemeinschaften strukturieren ebenso wie der resultierenden Muster ermöglichen. Abschließend kann festgehalten werden, dass Naturschutzmaßnahmen zu großen Teilen in den phylogenetisch diversen Urwaldrelikten konzentriert werden sollten, um sicher zu stellen, dass ein Maximum an Biodiversität erhalten bleibt.

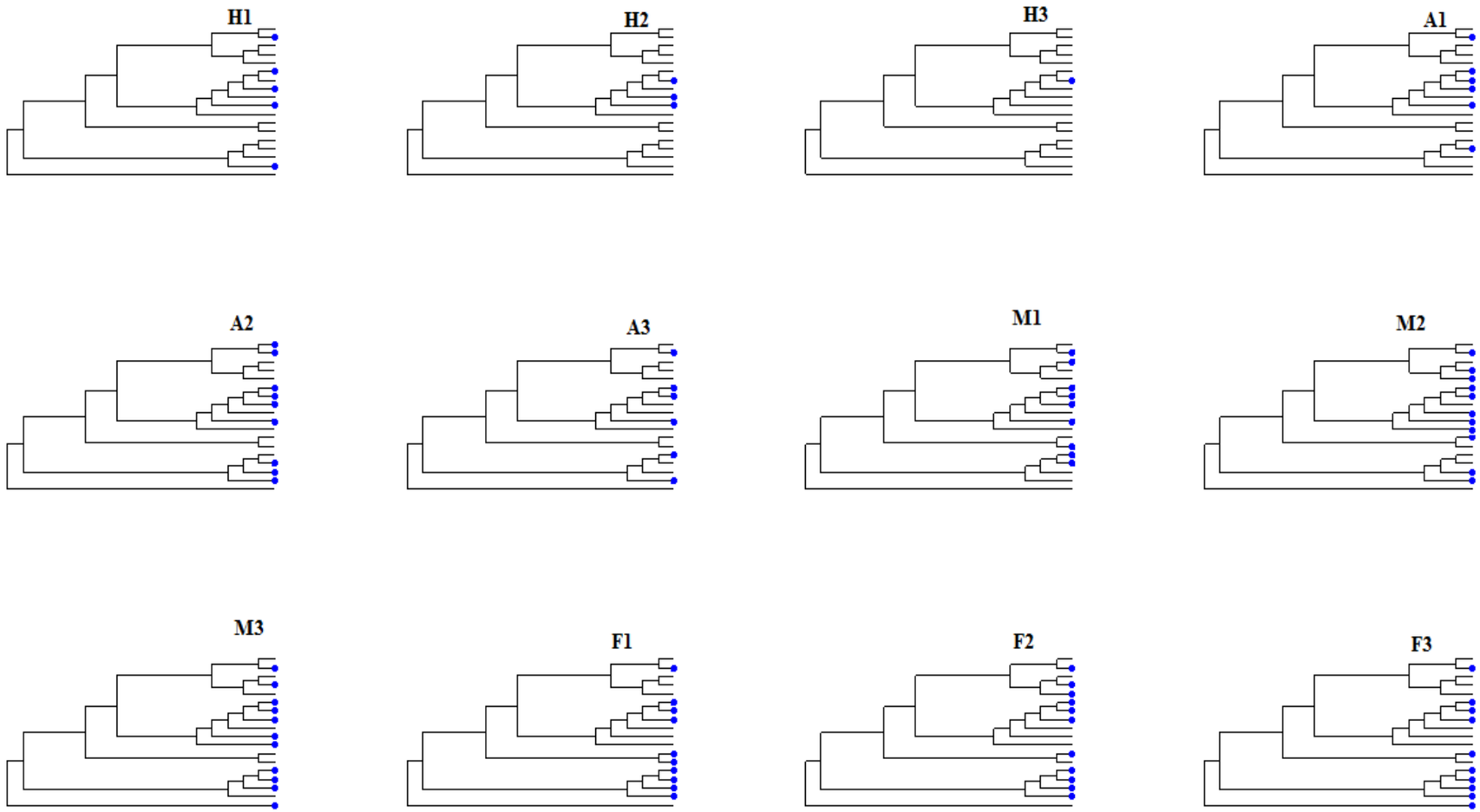
Appendices

7 Appendices

Appendix 1. Original distance matrix for between species phylogenetic distances used in constructing the initial phylogeny of ants in the genus *Pheidole*.

	G01	G07	G08	G12.	G22	G10	G15	G16	G14	G18	G23	G19	G05	G04	G02	G03	G11
G07	0.155																
G08	0.169	0.194															
G12.	0.196	0.214	0.200														
G22	0.242	0.232	0.207	0.211													
G10	0.207	0.190	0.223	0.210	0.196												
G15	0.181	0.173	0.213	0.194	0.231	0.164											
G16	0.199	0.226	0.220	0.228	0.206	0.189	0.201										
G14	0.216	0.230	0.212	0.216	0.209	0.197	0.225	0.174									
G18	0.215	0.206	0.234	0.207	0.221	0.192	0.195	0.205	0.185								
G23	0.220	0.206	0.236	0.209	0.228	0.192	0.199	0.193	0.193	0.155							
G19	0.203	0.193	0.242	0.202	0.219	0.185	0.190	0.189	0.199	0.157	0.156						
G05	0.185	0.200	0.223	0.204	0.217	0.195	0.183	0.191	0.188	0.221	0.217	0.188					
G04	0.201	0.218	0.218	0.209	0.224	0.190	0.197	0.197	0.174	0.201	0.199	0.200	0.191				
G02	0.184	0.200	0.216	0.212	0.208	0.166	0.191	0.176	0.160	0.200	0.199	0.199	0.171	0.157			
G03	0.232	0.198	0.242	0.231	0.212	0.202	0.204	0.196	0.184	0.227	0.227	0.215	0.173	0.169	0.142		
G11	0.200	0.208	0.223	0.204	0.225	0.188	0.212	0.203	0.188	0.214	0.231	0.229	0.202	0.193	0.198	0.211	
G13.	0.232	0.219	0.251	0.216	0.216	0.182	0.214	0.218	0.198	0.242	0.236	0.221	0.194	0.204	0.192	0.223	0.193

Appendix 2: Phylogenetic spread of species in the ant genus *Pheidole* in Rio Cachoeira Nature Reserve



Appendix 3. The table lists possible substitutions for genera that are not represented in the phylogeny of Brady et al. (2006). Substitutions are „closely related“genera. The definition of “closely related” here means that the original genus as well as the proposed substitution is a member of the same tribus. The assignment of genera into tribes follows Bolton 2003.

Original taxa	**Substitution**	**References**
Rogeria	Stenamma	Bolton 2003
Adelomyrmex	Stenamma	Bolton 2003
Lachnomyrmex	Stenamma	Bolton 2003
Octostruma	Basiceros or Eurhopalothrix	Bolton 2003
Carebarella	Solenopsis	Bolton 2003
Oxyepoecus	Solenopsis	Bolton 2003
Carebara (was: Oligomyrmex)	Solenopsis	Bolton 2003
Megalomyrmex	Solenopsis	Bolton 2003
Hylomyrma	Pogonomyrmex	Bolton 2003
Cyphomyrmex	Trachymyrmex	Bolton 2003; Wetterer et al. 1991

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