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Master's Program in Evolution, Ecology and Systematics

Speciation of Ants in the Tropics of South America

Master's Thesis

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…to my dad

Contents

Abstract

Mountain building exerts a large impact on landscape modification across time, but also it may promote speciation through vicariance. The influence of geographic isolation on the evolution of ants (Hymenoptera, Formicidae) fostered by the Andes uplift in northern South America, was evaluated in this study. Morphological observations on arboreal ants inhabiting pristine forests in two Ecuadorian megadiverse regions, Chocó and Amazonia, which are located at both sides of the mountain range, revealed various unusual cases of ant crypticism between these regions. Based on these novel records, I tested the hypothesis of ant speciation driven by vicariance, which should have generated morphological differentiation among these formicids after millions of years of being separated by the Cordillera. Molecular barcoding strongly supported the lineage split between most target species which was explained by their genetic distances. This evolutionary split was also supported by morphological phylogenies, though not for all the species. Divergence times estimations between Chocó and Amazonian ant lineages based on a relaxed fossil calibrated molecular clock, suggest that lineage bifurcations took place in the Neogene (ca. 20 to ca. 5 Ma) coinciding with a period of intense geologic activity and landscape rearrangement, promoted by Andean tectonics. In addition, I provide more arguments for the rising hypothesis that cryptic species in the Hymenoptera are more common than previously suggested, even when these are separated by large natural barriers. I propose new ant species in the genera *Camponotus*, *Dolichoderus*, *Ectatomma, Odontomachus*, and *Pachycondyla*.

Keywords: Amazonia; Andes uplift; Ants; Chocó; Cryptic species; Ecuador; Vicariance.

1. Introduction

Speciation is one of the most intriguing evolutionary processes which is directly responsible for the diversity of life (Gavrilets 2003). Understanding speciation still remains a big challenge faced by evolutionary biologists today. Classically, geographic speciation can be classified in three modes: allopatric, parapatric and sympatric (Dobzhansky 1951, Mayr 1963, Coyne and Orr 2004,) the first mode being the most common way for species to split, especially in animals (Hoskin *et al*. 2005). Under allopatric speciation, there is no migration of individuals (and gene flow) between diverging (sub) populations (Rivas 1964, Lande 1980, Gavrilets 2003, Campbell & Reese 2009). In the absence of gene flow, reproductive isolation arises gradually and incidentally as a result of mutation, genetic drift and the indirect effects of natural selection driving local adaptation (Dobzhansky 1951, Mayr 1963, Coyne and Orr 2004).

The overall geographic range of a species is determined largely by a series of historical accidents and a species will extend its range until it is stopped by barriers to dispersal (Slatkin 1987). Allopatric speciation is usually better discernible when considering natural barriers. Mountain ranges, deserts, oceans, and other major geographic features together form a network of barriers that separate potentially isolated regions (Slatkin 1987) and their inhabiting organisms. The northern Andes in South America are a well suited example depicting the natural process of geographic isolation and its sequent biological consequences on their animal and floral dwellers.

In north-west South America, two tropical regions with an extraordinary and unique biota are separated by the Andes mountain chain. The first one, to the Atlantic coast, comprises a biodiversity “hotspot” termed as Tumbes-Chocó-Magdalena (Chocó for short), one of the wettest places on Earth (Mayers *et al.* 2000, Olson *et al.* 2001, Conservation International 2012). The other one is the Western Amazon Basin, one of the world’s biologically richest regions today (Mayers 2000, Olson *et al.* 2001, Sarkar *et al.* 2008, Bass *et al.* 2010). Climate, vegetation and, in the end, biodiversity and the evolution of all life in both land areas is strongly influenced by this mountain range (Gregory-Wodzicki 2000). Mountains affect climate because they change patterns of precipitation and seasonal heating (Antonelli *et al.* 2009), act as a barrier to atmospheric circulation, may increase rates of chemical weathering (Ruddiman and Kutzbach 1989, Raymo and Ruddiman 1992, Hay 1996, Broccoli and Manabe 1997) and reorganize drainage patterns during the course of

tectonic events (Hoorn 1995). In fact, Raymo and Ruddiman (1992) proposed mountain building as the culprit for the marked global-cooling trend observed since the Eocene.

Although current geological and climate conditions in both tropical regions are relatively similar, in the past major geological events such as the uplift of the Andes and marine incursions from the Pacific and the Atlantic (Nuttal 1990, Hoorn 1993, Hoorn *et al.* 1995, Steinmann 1999, Santos *et al.* 2008,) dramatically shaped the landscape of north-central South America. During those processes migration pathways as well as natural barriers were progressively formed, fostering the dispersal and adaptation of the organisms to their newly formed habitats (Vrba 1992, Antonelli et al. 2009), ultimately these environmental stresses induced species differentiation (Sheldon 1996, Renaud and Dam 2002).

The present investigation focuses on the speciation events that have occurred by vicariance as a result of the isolation of the Chocó and Amazonian organismic populations located on both sides of the Andes mountain range in the geographical area of Ecuador. The ants (Hymenoptera, Formicidae) comprising a conspicuous, hyperdiverse, and thoroughly studied terrestrial invertebrate group (Hölldobler and Wilson 1990, Kim 1993, Basset *et al.* 1998, Davidson *et al.* 2003) will serve as model for this purpose. To my knowledge, this study is the first attempt to evaluate the isolation effects that large natural barriers and its past geologic history had on the evolution of ants in northern South America. Results of this investigation provide new information to the systematics and biogeography of Neotropical formicids, adding new insights on their evolution as well. Through this work I hope to promote further research on insect evolution in South America, but also in parallel, enhance our current taxonomic knowledge on the inmense diversity hosted in the New World Tropics.

1.1 Background

The main hypothesis of this study, which is, that remote tropical pristine forests located in Ecuadorian Chocó and Amazonia regions may share several ant species despite being geographically separated by the Andes Cordillera, arose from repeated observations made during taxonomic determinations of arboreal ants, which were previously collected by the author and a team of collaborators, at the mentioned regions during 2001 and 2007. Several dozens of canopy samples, each containing various thousands of arthropods,

were examined in order to extract all the ants contained in there for posterior taxonomic work. After comparing the specimens from several samples, it was found at the time that some of them apparently occur in both tropical regions (unpublished). The rationale behind this assumption is that little or no morphological variation between these specimens was found. Further deeper analyses were pending.

Around two hundred samples were collected by insecticidal canopy knockdown during the mentioned period, following suggested methodologies by Roberts (1973) and Erwin (1983). These collections were deposited at the Museo de Historia Natural "Gustavo Orcés V.", Escuela Politécnica Nacional University (MEPN) in Quito - Ecuador, where the first assumptions regarding the mentioned hypothesis were made.

Sampling locations at Chocó and Amazonia are separated by around 400 km (Appendix 5). Today the Andes mountain chain is the only large natural barrier present between them. The highest geographical locations across the West-Northern Andes reach elevations of ca. 2500m (North of Perú, South of Ecuador and Southern most part of Colombia), to ca. 5000 m (Central-Northern Ecuador) (Van der Hammen 1987, Valencia *et al.* 1999, Gregory-Wodzicki 2000). Access to each location required a great effort in managing logistics as well as dealing with time-consuming negotiations with native communities, NGOs and governmental organizations. All sampling localities (see Apendix 1) in both regions are virtually isolated from disturbance activities related to large human settlements as for example the capitals of nearby provinces. There are no motorized roads leading to the sampling sites in Chocó, and the only way to get to these places is by canoe, whereas localities in Amazonia are relatively more accessible through third-order (ballasted) roads, however special research permits are required.

The MEPN entomological collections hosts specimens representing a broad variety of terrestrial habitats in Ecuador belonging from many localities, from tropical lowland rainforests to high elevation cloud forests, to Andean dry forests, embracing an altitude range from ~ 100 to > 3000 m. In past years, a variety of ant specimens were examined by the author and collaborators from numerous sample sets. A discernible pattern of species vertical distribution was inferred (not published), this is, many ant communities inhabiting tropical lowland forests are not found in high altitude forests (ca. 2000 m to > 3000 m) and vice versa. A case depicting this argument is provided for example, by field collections and observations of many tropical ponerine ants (subfamily Ponerinae) like those from the

genus *Pachycondyla*, e.g., *P. carinulata* (Appendix. 2Q), *P. inversa* (Appendix. 2S) (included in this study) which have been found in several tropical habitats in locations up to 2000 m (Mackay and Mackay 2010, Longino 2010), but never in high altitude montane habitats like temperate Andean forests which are composed by quite different vegetation (Valencia *et al* 1999). These arguments have direct implications on the hypothesis proposed for this study since is not likely that tropical ant species adapted to lowland or even montane habitats below ~ 2000 m extend their distribution to high-altitude habitats. Based on that, gene flow restriction between Chocó and Amazonian ant populations is plenty possible. Nonetheless, among others, a critical point to consider is the latitudinal geographic distribution of the species selected for analyses which is to be discussed later in this manuscript.

2. Materials and Methods

2.1 About the Species and the Specimens

The data base of the MEPN Entomology Section was revised so as to locate the target set of samples and localities per region available for this study. Starting from this, four localities from Chocó and nine from Amazonia were selected (see Appendix 5). Based on what had been done before, the main objective during this step of the research was to locate and identify 10 specimens from each target species (Table 1) deposited in the MEPN collections. This "required" number of specimens/species was randomly set as a confidence bound so that, after a second identification round, discarding wrong determined specimens, it is ensured that a minimum of five identical specimens/species/region are retained for posterior analyses: three for identifying and coding character states during the morphological phylogenetic approach, and two specimens for DNA analyses.

According to the stated hypothesis, the aim was to find specimens belonging to "the same" species in both Chocó and Amazonia, that is, they had to be morphologically identical, or as a minimum very similar, but without considering two aspects that are usually taxonomic pitfalls in ant identifications: i) plastic characters, like the number of mandibular teeth (e.g. in the genus *Pachycondyla)*, or specific number of hairs in the body and sometimes body color; and ii) caste dimorphism and polymorphism, which is very frequently a cause of miss-identification due to morphological variation mainly between workers of some genera like *Camponotus* - carpenter ants - (Fernández 2002, Fernández 2003), which in some

cases show up to 4 distinct variations among the worker caste (*pers. obs.*). During the extraction process, the specimens found in one region were constantly compared to their species counterparts found in the other region so as to have confidence in obtaining the correct material.

Finally I revised initial identifications for all the species extracted, aided by dichotomic keys from Mackay (1993) for the genus *Dolichoderus*; De Andrade and Baroni Urbani (1999) for *Cephalotes*; Fernández (2007) for *Odontomachus*, *Paraponera*, *Platythyrea*; Mackay (2007) for *Camponotus*; and Mackay 2010 for *Pachycondyla*. Digital images posted in authoritative internet sources were an additional support as Ant Web (www.antweb.org) and Ants of Costa Rica maintained by Longino (2010). Close to hundred canopy samples were revised at the end of this phase, being 30 of those inspected (concerning ants) for the first time since they were collected in the field ca.14 years ago.

The species used for the analyses are indicated in Table 1. It is also shown the region were they were collected, number of specimens per species used in each approach (morphological and molecular), and the MEPN's voucher code. All individuals per species belong to the same worker caste. For all the species up to three individuals were used for DNA isolation, PCR and sequencing, however I decided to show only the number of specimens which results were completely analyzed just before writing this manuscript (further details are provided in *molecular analyses and phylogenetic inference*).

Species included in the genus *Platythyrea*, *P. angusta*, collected in Amazonia, and *P. pilosusla* from the Chocó region, were initially considered (during the extraction process) as belonging to a single species, recognized as *P. angusta*. Later, during the morphological analyses it was classified in those species mentioned before. This is supported by non-phylogenetic taxonomy in Fernández (2007).This is the only genus among the target set of taxa for which two species are morphologically confirmed as separated entities, prior to obtaining the final results. In any case, it was added to the data matrix for morphological analyses and also included for DNA sequencing as the status of these two species has not been confirmed by any of these methodologies, until today.

Table 1. Ant species examined in this study. Under "*Chocó" and "Amazonia*" columns: numbers to the left of the slash are specimens used in the morphological analyses, to the right of the slash specimens used in molecular analyses (*cox1* gene). Specimens obtained from more than one sampling site (several MEPN vouchers) are represented with an asterisk. The complete set of vouchers is shown in Apendix 1. See details in the text regarding blank spaces in *Platythyrea angusta* and *P. pilosula*. DNA vouchers are deposited in the ZSM DNA Bank.

Subfamily	Species	Chocó	Amazonia	MEPN Voucher
Formicinae	*Camponotus sericeiventris*	5/1	4/1	1559/1421
	Camponotus sp. 1	3/1	5/1	*/*
	Camponotus sp. 2	5/1	6/1	1557/*
	Camponotus sp. 3	4/1	3/1	1037/1361
	Camponotus sp. 4	4/1	5/1	1556/1421
	Camponotus sp. 5	3/1	4/1	1556/1361
	Camponotus sp. 6	3/2	2/2	*/*
	Camponotus sp. 7	5/1	3/1	1568/1313
	Camponotus sp. 8	5/1	6/1	1570/1445
Myrmicinae	*Cephalotes basalis*	1/1	1/0	*/3711
	Cephalotes maculatus	5/1	2/1	1564/*
Dolichoderinae	*Dolichoderus superaculus*	5/1	2/1	1568/1361
	Dolichoderus sp. 1	5/2	4/2	1557/1281
	Dolichoderus sp. 2	5/1	6/1	1559/1421
Ectatomminae	*Ectatomma tuberculatum*	5/2	3/1	1557/*
Ponerinae	*Odontomachus hastatus*	3/1	1/1	*/1421
	Pachycondyla carinulata	6/2	4/1	1556/1295
	Pachycondyla crenata	5/1	5/1	1556/*
	Pachycondyla inversa	2/1	4/1	*/1295
	Pachycondyla sp.	1/1	4/1	*/1446
	Platythyrea angusta		3/1	1299
	Platythyrea pilosula	1/0		4833
Paraponerinae	*Paraponera clavata*	4/1	5/1	1563/1093

2.2 Molecular Lab Methods

Before examining the set of specimens brought from Ecuador, several DNA amplification tests were carried out in the Population Genetics Lab. of Prof. Wolfgang Stephan at the Department of Biology, Biocenter II, Ludwig Maximilians Universität (LMU) Munich, during February - March 2012. Several fresh ants from the species *Formica* sp. and *Tetramorium*

sp. collected by the author in Munich, as well as four-year-old dried specimens from the genus *Leptothorax* provided by Prof. Volker Witte from the Behavioral Ecology group LMU, were used in the tests.

DNA Isolation, Amplification and Sequencing

This part of the molecular work was performed at the Lab facilities of the Zoologische Staatssammlung München (ZSM) Munich - Germany.

Puregene Core Kit A (Qiagen Sciences, USA) DNA extraction kit was used to obtain pure DNA from a total of 48 ant specimens (Table 1). Prior to this step the ants were observed under a stereo microscope searching for possible ectoparasites as e.g. acari, which are very common among ants. One or two legs were removed from each specimen and placed inside a sterilized 1.5 ml Eppendorf tube. In the case of tiny specimens like *Cephalotes maculatus* (Appendix 2J) the entire thorax was used for analyses. The tubes containing the specimens where placed in a special tank with liquid nitrogen, 5 tubes each time, for ca. 15 minutes. This time lapse is sufficient to freeze the ant's cuticle at the point of getting it completely broken when making the grinding step. Immediately after taking the tubes out of the tank, 100 ul of protein lysis solution were added in each tube to start grinding the ant parts with plastic sterilized pestles. Then, 0.5 ul of proteinase K were added in each tube and placed the whole set in waterbath preset at 56 °C for overnight incubation.

Posteriorly, 35 ul of protein precipitation were added to the samples, centrifuged for 3 minutes at 13.000 rpm, carefully extracted the supernatant containing the DNA with a micropipette and placed this solution in a new 1.5 ml Eppendorf tube. For the DNA cleaning, 150 ul of isopropanol were added and then centrifuged for 3 minutes at 13.000 rpm, afterwards the flow-through was discarded, and 150 ul of 70% ETOH were added to "wash" the DNA residuals. After a 3 minute centrifugation at 13.000 rpm the ETOH was discarded and the tubes (with the lids opened) placed to dry at room temperature for a few minutes. Finally the DNA was eluted in 30 ul of ddH2O, afterwards measured in a NanoDrop 1000 Spectrophotometer (Thermo Scientific Inc.), and stored at -4 °C to further analyses.

The nuclear mitochondrial gene that encondes for the enzyme cytochrome *c* oxidase subunit one (*cox1*) was amplified in this study. This gene was chosen among others (e.g. *cox2*, *cob*,*16S*, *s8S*, usually selected for phylogeny inference) for two reasons which

alternatively match the objectives that I pursue through this study. First, *cox1* primers used here (see below) enable recovery of this gene's *5'* end from representatives of most animal phyla (Folmer et al 1994, Zhang and Hewitt 1997) and has been used intensively in arthropod molecular taxonomy (Chen et al 2000, Besansky et al 2003, Hebert and Ryan Gregory 2005, Savolainen *et al.* 2005, Schindel and Miller 2005, Costa *et al.* 2006) as well as in Formicidae research (Smith *et al.* 2005). Second, the evolution of this gene is rapid enough to allow the discrimination of not only closely allied species, but also phylogeographic groups within a single species (Cox and Hebert 2001, Wares and Cunningham 2001). Finally, *cox1* could be the most suitable locus for times divergence estimations according to thorough assessments by Gaunt and Miles (2002)

Cox1 amplification was carried out according to a standardized "two-step" PCR protocol employed at the ZSM's molecular Lab as follows, first step: 1 min/94 °C; 5 cycles [30 sec/94 °C; 40 sec/47 °C; 10 min/72 °C]; second step: 30 sec /94 °C; 30 cycles [40 sec/52 °C; 1 min /72 °C; 10 min/72 °C]; 10 min pause. Forward primer LCO1490 5'-GGT CAA CAA ATC ATA AAG ATA TTGG-3' and reverse HC02198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' from Folmer *et al.* (1994) were used in the following reaction mixes (master mix) per DNA sample: 13 ul ddH2O, 5 ul of Mango buffer (5x reaction red colored – Bioline), 1.25 ul MgCl2 (50 mM – Bioline, Germany), 2 ul dNTPs (10 mM – Bioline, Germany), 1 ul of each primer (5pm/ul), and 0.5 ul of mango *Taq*-DNA-Polymerase (Bioline, Germany). Twenty three ul of this reaction mix was added to each PCR tube plus 2 ul of template DNA from each of the 48 specimens shown above. After a brief vortex the mix was placed in a Biometra thermocycler (Biometra, Germany) following the protocol described before. Two ul of each amplicon were placed in agarose gel (GTQ agarose – Roth, Germany) for a further 20 minute electrophoresis run so as to test the presence of the expected gen product (ca. 600 bp.), as well as to recognize possible nuclear pseudogenes of mitochondrial origin (*numts*, López *et al.* 1994).

PCR clean-up was carried out by adding 2.5 ul of 3 M sodium acetate (pH 5.5) plus 25 ul of cold 100% ETOH to each amplicon, then centrifuged for 30 min at 3600 rpm, discarded the supernatant and remotion of residuals by 1 minute centrifuge of the inverted plate with the amplicons covered with sterilized absorbent paper. In a second cleaning process, 25 ul of 70% ETOH were added to each well, and the same following steps as described above were made, including a complete dried of ETOH residuals.

Two reaction mixes were prepared for cycle sequencing, each containing a single primer to match a DNA strand (forward and reverse) of the amplified gene, as follows (quantities/sample): 4.05 ul ddH2O, 0.5 ul of sequence buffer (ABI), 0.3 ul Big Dye 1.1 (ABI) and 1 ul of each primer. One ul of the cleaned amplicon was added to the resulting 5 ul of this reaction mix in each well, getting in total 48 samples/per primer (1 sequencing plate), which then was placed in the thermocycler following a Big Dye standard protocol.

For the cycle sequencing clean up, a mix of 3200 ul 85% ETOH plus 250 ul of Cleanseq solution (Angecourt/Beckman Coulter Genomics) was prepared, out of which 33.5 ul were added in each well containing the cycle sequencing product. Following a brief vortex the complete plate was then allocated for 1 hour in a magnetic rack whose function is to retain the amplicon attached to its walls by means of “metallic beads” contained in the Clenseq solution. In the next step this solution was removed carefully with a pipette and then 100 ul of 85% ETOH were added to each well so as to remove non-required cleaning residuals, after 5 minutes the ETOH was extracted, oven-dried for ca. 1 minute and finally sealed with special plastic adhesive for further sequencing which was performed at the Biocenter LMU sequencing facilities,.

2.3 Molecular Analyses and Phylogenetic Inference

Forward and reverse *cox1* sequences were edited using the programs BIOEDIT (Hall 1999) and SEQUENCHER 4.10.1 (Gene Codes, Ann Arbor, USA). NCBI *blast* tool (www.ncbi.nlm.nih.gov), the Barcode of Life - BOLD Systems (www.boldsystems.org), and the Formicidae Barcode of life (http://formicidaebol.org) digital data bases were employed to check/verify whether the resulting *contig* sequences match ant species sequences already stored in these sources, as well as checking for possible contamination resulting from previous molecular procedures.

Outgroup selection was inferred from studies of Brothers and Carpenter (1993), Grimaldi and Engel (2005), Moreau *et al.* (2006), Rabeling et al. (2008), and Heraty *et al.* (2011) where analyses of Hymenopteran evolution were carried out including Formicidae. In addition, prior Maximum Likelihood (ML) tree reconstructions were made, by removing and adding species outside the Formicidae, so as to check for suitable taxa in order to root the species ingroup (ants) being analyzed. Among the species tested, *Atomacera debilis* (Argidae) and *Dasymutilla sp.* (Mutillidae) proved to fit well, that is, the species ingroup remained consistent and relatively well supported with, or without including them in the

phylogenetic reconstructions. *Dasymutilla* in the family Mutillidae, along with other *aculeate* (stinging Hymenoptera) families like Scoliidae or Pompilidae are considered today sister groups to Formicidae (Heraty *et al.* 2011). Whereas species in Argidae (sawflies) are far less related to the Formicids, being considered among the basal Hymenoptera. Sequences of both species were obtained through Genbank, accession numbers GQ374618 and AF142531 for *A. debilis* and *Dasymutilla* sp. respectively.

Although in most of the species, three specimens were used for molecular analyses the majority of the results in this study are represented by one specimen, excepting six species for which two individuals were ready for analyses (Table 1). In contrast, no good DNA was obtained from *Platythyrea pilosula* and *Cephalotes basalis* for which just one specimen was available.

DNA isolation, PCR and sequencing were executed in separate steps, including 48 specimens each time for allocation in a 96-well sequencing plate (primers forward and reverse), thus avoiding unnecessary waste of time and materials in case of failure. An extended analysis using the results of the remaining molecular samples is to be presented in another study.

Despite the antiquity of the samples, 33 sequences were successful (68%), each *contig* ranging in quality from 50% to 98%. Given this, it was not possible to get an equal number of sequences/species/region (the optimum), that is, both regions were unevenly represented by each species, for example sequences of *Pachycondyla inversa* were only obtained for Chocó preventing further comparisons with its similar species from Amazonia. To avoid this problem I searched in Genbank and in the Barcode of Life sources, unfortunately just two species, *Paraponera clavata* and *Camponotus sericeiventris*, are currently available there. Field collection data of the latter could not be confirmed, leaving just *P. clavata* (collected in Costa Rica, accession number GQ374640), as the only possible *cox1* sequence to be used. Therefore, I omitted from the molecular analyses the following species: *Camponotus* sp. 1, *C*. sp. 2, *C*. sp. 3, *C*. sp. 5, *C*. sp. 8, *Dolichoderus superaculus*, *D*. sp. 2, *Odontomachus hastatus*, *Pachycondyla inversa*, *P. crenata*, *P*. sp.1, *Platythyrea pilosula* and *P. angusta*. The remaining 10 species were used for all further molecular analyses.

Sequence alignments were performed with MEGA5 program (Tamura et al. 2011) employing CLUSTAL W (Thompson *et al.*1994) and MUSCLE (Robert 2004) algorithms.

Alignment errors were inspected and corrected by hand in MEGA5, simultaneously checking the chromatograms in SEQUENCHER. A total of 143 ambiguously aligned sites were removed, obtaining a final sequence block composed of 677 bp. Upon completed the alignment the data set was translated into amino acid sequences to check for stop codons, when present.

Phylogenies were inferred under Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian approaches. Regarding MP, the data set was analyzed in the program TNT vr. 1.1 (Goloboff *et al.* 2008), first setting the outgroups and then running several rounds of Jacknife resamplings each consisting of 1,000 replicates with a maximum storage of 20,000 trees in memory, under the "new technologies" option, including the algorithms *sectorial search*, *ratchet* and *tree fusing* which provide a fast and efficient *via* for analyzing data under parsimony (see more details about these algorithms under *Morphological Phylogenetic Inference* in this manuscript). After the first round of replications a "best score" (tree length) is given, this value was then used as a starting score bound for the next jackknife sampling rounds but this time, telling the program to "give up" after being found an even greater value than the first best score, thus ensuring that an optimum is reached within the searchable tree space. A random search starting "seed" was set every sampling round. After obtaining the most parsimonious trees, a majority consensus and strict consensus trees were calculated and compared. Afterwards the resulting final tree was annotated with Bremer support (or decay index) values (Bremer 1994).

For ML and Bayesian analyses the sequences set was partitioned using MESQUITE program (Maddison and Maddison 2011) so as to compare phylogenies based on the evolutionary rate of each codon position. In addition, a suitable model of nucleotide evolution was statistically searched based on the AIC and BIC criteria using the program JMODEL TEST (Posada *et al*. 2008).

Phylogeny inference under ML was made with the program RAxML 7.0.4 (Stamatakis *et al.* 2008) using partitioned alignment data as indicated above. The number of bootstrap replications was fixed to 3 $x10^7$ with default program settings.

Under the Bayesian approach using MRBAYES 3.1 program (Ronquist and Huelsenbeck 2003) each partition was tested separately, being the three partitions: i) codons 1-2 versus codon 3, ii) codon 1 versus codon 2, and iii) a partition where each of the three codon positions was compared. Model HKY+G (Hasegawa *et al.* 1985) was selected for the first

partition, while GTR+I+G (Tavaré 1986) was selected for the next two partitions. Two Marcov chain Monte Carlo (MCMC) runs were executed, each with four chains with a total of 3 x 10^7 generations (cycles) with a sample frequency of 1000 generations, setting to six the number of nucleotide substitutions. By default 34 parameters of evolution were tested for each partition by means of the Monte Carlo method (Metropolis and Ulam 1949) as implemented in MRBAYES through the MCMC algorithm, which searches for the trees with the highest posterior probability density. Upon confirming that both MCMC runs converged into a stationary distribution (PSRF ~ 1) meaning that most of the tree space was searched, a majority consensus tree was calculated after discarding 25% of the initial inaccurate trees with a *burnin* of 7,500 out of 30,000 possible trees. The tree topology resulting from partition (iii) was selected as it showed the best harmonic distribution mean value (LnL= -5976, SD± 0.17) as inferred from the marginal likelihood test (Suchard *et al.* 2001).

2.4 Morphological Phylogenetic Inference

Following the classic approach in modern parsimony analyses, a coded morphological matrix (Apendix 3) composed of 98 characters was used to infer evolutionary relationships among the target species. In total 167 ant specimens belonging to the worker caste were examined, most of the species were represented by 3 to 5 individuals (Table 1), which were collected in different localities from different ant colonies. Character coding was based on a comparative anatomical study of the specimens by means of direct observation. Species in this study are treated as terminal taxa, also called the exemplar approach, which is considered superior than the supraspecific ground plan (see Prendini 2001 for a review) which was used for example by Baroni Urbani (1989) in the first attempt of elucidating a morphological phylogeny of ants by means of quantitative methods. The use of exemplar species as terminals is justified as it enhances documentation, maintenance and continuity of morphological data through time (Keller 2011), in the same way the compilation of DNA sequences is based mainly on the use of species as the units for molecular studies (Vrana and Wheeler 1992).

Twenty one characters were considered additive as inferred from the evolutionary steps (whatever necessary) involved in the morphological transition from a given structure, to another, without making *a priori* assumptions regarding ancestral or more recent forms.

Dollo characters, though probably identified in this study, are not considered due to lack of reliable information.

Thirty seven characters are coded for the first time, some of which may play an important role for determining taxonomic boundaries between tribes and genera. One example is a row of minute denticles located in the basal mandibular margin of examined species in the genus *Dolichoderus* (see Char. 26, Appendix 2: L,M,N), which presumably is a trait with high potential as a source for homology within this genus. Another set of 33 characters revised from currently relevant and updated literature regarding ant taxonomy, biogeography and evolution, were analyzed and its suitability was tested and implemented on the species examined in this study. In some cases this required the reinterpretation of taxonomic definitions aimed to describe synapomorphies for a specific formicid clade (e.g. the army ants, in Ward and Brady 2003) and further modification of the character definition to be allocated in the species examined here. Finally, the remaining 28 characters were adopted from the literature and implemented in the current study without modifications, as they fit properly in the data set describing the variation of derived traits within species.

The same outgroup species used for the molecular phylogenies were also employed here, so as to facilitate further interpretations and comparisons between both approaches. As stated by Keller (2011) choosing nonformicid outgroups can post important problems during the search for homologies and posterior tree reconstruction, because the wingless worker caste is an evolutionary novelty among the Hymenoptera, so in principle there is no comparable semaphoront outside the Formicidae. Winglessness is present also in other hymenopteran families (e.g. Mutilidae, Tiphiidae) but this is not a result of polyphenism thus, winglessness in worker ants would not be a proper character for evolutionary comparisons to those families. Nevertheless, besides the nonflying thoracic sclerites and visual organs (e.g. oscelli, and compound eyes) most of the remaining external skeletal structures are comparable between worker ants and flying females (Keller 2011).

To facilitate posterior evaluations of character states and their definitions presented here, each character treatment comes with a reference of the author(s) who either coded that character for the first time or used it as part of another study. Insofar as possible, I added to most character descriptions an example of the species, genus or subfamily examined here which exemplifies a given character state. In several cases, especially those in which a character has been considered important in defining species boundaries (e.g.between

cryptic species), is also possible to visualize in this manuscript character state definitions through a series of scanning electron microscope (SEM) images obtained with a standard LEO 1430 VP SEM at the ZSM during the taxonomic work. High quality digital photographs taken in profile view of each species were included as well (Apendix 2).

Characters Surveyed

The three somatic parts of the ants body were scrutinized and compared among species including the outgroups. In the prosoma (head) structures like the antennae, antenomeres, eyes, mandibles, labial and maxillary palpi were observed. In the mesosoma (thorax), some of the body features examined were the pronotum, propodeum, mesosomal sclerites, the legs and their compounding structures. Finally in the metasoma (abdomen), the sting, acidopore, petiole and petiolar components, abdominal constrictions among several other characters were analyzed. In addition, the amount of pilosity, setae, spurs, cleaning structures, rugosities and cuticular sculpture, location and orientation of glandular structures, vestigial divisions between sclerites of the body, were observed and coded in most of the mentioned structures. Although an intense and detailed work has been done translating corporal features into numerical characters representing homologous structures among the set of formicids selected for this study, there is still a broad amount of morphological characters, either externally as well as internally, to be considered as a source for synapomorphies.

2.4.1 Cladistic Analyses

In order to make a proper comparison with the results obtained *via* molecular analyses, a second reduced matrix containing 87 characters was analysed. The reason for this (as detailed before) is that the molecular phylogeny is composed of just 10 species (out of 23), thus, it was required to remove the morphological characters only related to those omitted species, avoiding this way uninformative sites in the matrix.

Analyses were made under parsimony as it implements a model that seeks to maximize homology from the primary conjectures coded in the matrix to an optimal (Farris 1983, de Laet 2005) or at least, a near-to-optimal phylogeny. I chose to work with the program TNT vr. 1.1 (Goloboff *et al.* 2008) because it effectively enhances the speed of phylogeny inference under parsimony showing an exceptional promise especially for analyses of large data sets (Hovenkamp 2004; Giribet 2005; Meier and Ali 2005; Goloboff and Pol

2007) through the implementation of new algorithms - in reference to those used in PAUP* (Swofford 2001), for example - that perform a thorough evaluation of the tree space (Nixon 1999, Blair and Murphy 2011). In this sense, I tested the performance of the so called "new technology search" algorithms, *Sectorial Search*, *Parsimony Ratchet* and *Tree Fusing*, using both implied and equal weights on the characters.

Unless stated otherwise, the following information about the algorithms used here were taken from Goloboff (1999), and Nixon (1999). The settings implemented in each algorithm were chosen according to how well these performed during prior tests in this study. *Sectorial Search* creates reduced data sets from parts of the tree using hypothetical taxonomic units (HTUs), then analyses them and if a better solution (clade arrangement) is found, reinserts it into the tree topology. After several changes in the tree topology a global round of tree branch reconnection (TBR) is done to insure global optimality. This algorithm uses three main methods of which I used the *random sectorial search* (RSS) and the *exclusive sectorial search* (XSS), because these fit the data set the best. The first one selects and searches sectors at random in the tree space, while the latter goes beyond by analyzing every tree sector with no overlap ensuring that no part of the tree space is left unanalyzed. The *Parsimony Ratchet* alternate phases of perturbation during the search, this is, duplicates or deletes characters until a certain number of rearrangements is done. The phase containing optimal trees of equal height is retained in the middle of the disturbed area, until all rearrangements are done or a certain proportion of tree swapping is completed. Finally, during *Tree Fusing* several sub-optimal trees are mixed among each other by inserting all the clades of the source tree into the target tree, if it produces a better (more parsimonious) tree, the exchange is accepted. Rounds of tree fussing are fixed by the user, in this case I chose to run 10 rounds with the option of "dump fused trees" which prevents "clogging" by discarding suboptimal trees that accumulate in memory after all rounds have been made.

Both matrices with 98 and 87 characters either using equal weights or implied weights were analyzed with the same parameter settings discussed above, using the following structure: sectorial search with default values and discarding rearrangements of equal score; parsimony ratchet using 100 iterations and defaults in the remaining options; five rounds of tree fusing using posterior swapping after branch exchanging. After getting the first most parsimonious score (tree length) this value was set as bound for posterior tree searches, meaning that higher values (less parsimonious trees) would not be accepted.

Several runs were executed using a random starting seed, fixing to 10 the number of times that a given most parsimonious topology would be found by the program during each run. Resamplings were made with Jackknife using 100 replicates.

Additionally, implied-weighted characters were analyzed following the concavity function first introduced by Farris (1969), enhanced by Goloboff (1993) and later implemented in TNT by the latter author. The concavity function *K* down-weights homoplastic characters favoring those very reliable (with no homoplasy) according to a degree of strictness which can be from 1 onwards, where 1 is the strictest measure, giving high weights to the most reliable characters and down-weighting mediocre or unreliable ones (see Farris 1969 and Goloboff 1993 for an extensive revision).

2.5 Estimation of Divergence Times

On the grounds of the results obtained through the partitioned analyses executed in MRBAYES, the files containing the partition runs were visualized and analyzed in TRACER (Rambaut and Drummond 2007) so as to obtain statistical estimates of the highest supported tree topology by means of the marginal likelihood method (Newton and Raftery 1994) with smoothed estimation using a resampling of 1000 bootstrap replicates. As expected, partition (iii) (see above) was the best supported phylogeny by the Log likelihood function for the Bayesian posterior probabilities (Fig. 2).

Mutation rate consistency among lineages was tested with the likelihood ration test LRT ($2\Delta L$ = n-2, Felsenstein 1981), where *n* is the number of taxa, and *L* the negative log likelihood score. If a constant rate of evolution occurs between lineages, then the sequence data represented in a tree, where branch length differences represent sequence divergence, should not be significantly different from an ultrametric tree, where the tips of the tree are equidistant from the root (Gaunt and Miles 2002). Under the Maximum Likelihood analysis performed in MEGA 5, the null hypothesis of equal evolutionary rates throughout the tree was rejected for the present data set (LRT = 41.8; df= 23; $p< 0.01$), given this, it was necessary to adopt a relaxed molecular clock assumption under the Bayesian method, which allows for a continuous rate change across the branches in a tree (Thorne *et al.* 1998, Aris-Brosou and Yang 2002), this model has shown to outperform strict correlated models of evolution (Drumond *et al.* 2006) where mutation rate is consistent across time in all the species considered.

An “xml” file was created in BEAUTI (Drummond and Rambaut 2007) containing the information required by BEAST (Drummond and Rambaut 2007) to estimate divergence times given the molecular data set. This included setting priors in selected tree branches where some knowledge is known about their ancestral lineages. In this study I used ant fossils to constrain four of the six formicid clades represented in the data by the genera, *Camponotus*, *Cephalotes*, *Dolichoderus* and *Pachycondyla*, which contain also the majority of the species analyzed here (Fig. 4). The fossils are as follows: a *Camponotus* specimen from the Eocene Baltic Amber, approx. age 44.1 Ma (Dlussky 1997); a *Cephalotes* specimen from the Miocene Mexican amber, Mexico approx. age 15 to 20 Ma (de Andrade and baroni Urbani 1999); and two *Dolichoderus* and *Pachycondyla* specimens both from the Eocene at the Green River Formation, USA, approx. age 48.5 to 53.5 Ma (Dlussky and Rasnitsyn 2003). Finally, two Bayesian independent MCMC runs each consisting of 1.5 x10^6 cycles both under the tree prior of a simple *Yule birth* model of speciation (Harding 1971, Steel and McKenzie 2001), using the molecular clock approach of a relaxed log normal uncorrelated distribution among branches, together with a GTR+I+G model of site substitution with 6 categories (same as for the Bayesian runs in MRBAYES), were executed in BEAST. Log output parameters were checked continuously in TRACER to visualize MCMC convergence on the posterior distribution.

3. Results

3.1 Morphological Character Descriptions

Several of the following character states coded here include taxonomic discussions, thus allowing the reader a direct, and where possible, enhanced comprehension of the definitions made in each one. For an extensive and more detailed explanation of most of the characters been coded here, the reader may refer to Ward and Brady (2003), Brady and Ward (2005) and Keller (2011).

The following abbreviations regarding the "positional views" for character evaluation are used, each one in uppercases appearing immediately after the character title: dorsal (D), frontal (F), lateral (L), ventral (V), profile (P), entire body (EB), postero-dorsal (Pd). When combined positional views are suggested, e.g. dorsal and fronto-lateral view, then it follows: D/FL. When an "*" is indicated after a character title it means that it is coded for the first time. Some character states are illustrated with scanning electron microscope images refered in the text as SEM figures.

Except for few cases, instead of using measurements to describe character states (e.g. Ward and Brady 2003) I coded these by making reference to another structure, for example, the *posterior length of the frontal carina* (char. 11) is coded relative to the length of the eye, which is very close to that structure (when applicable). The use of this approach avoids ambiguities when for example, measuring the postpetiolar ratio in ants: "postpetiole width/gaster width ~0.78 [mm]" (Ward and Brady 2003: 370). That method restricts the comparison of homologous structures to a narrow range of individuals even of the same species, as in ants for example morphological intra-caste variation is quite common especially in species with polymorphic workers (Wilson 1953, Oster and Wilson 1978, Hölldobler and Wilson 1990). Furthermore, is much easier to compare structures using proportions and then refer them to nearby characters, instead of using strict ratios in tiny scales.

Finally, I will not show here a figure depicting common morphological features of a formicid, as there is plenty of related information in the literature. For that, the reader may refer to the following excellent internet sources (to name just a few): Hymenoptera Anatomy Ontology (www.glossary.hymao.org); Glossary of Ants RBINS Ant eMuseum (http://projects. biodiversity.be/ants); Ant (wikipedia.org). In addition, the work

“Identification guide to the ant genera of the world” by Bolton (1994) is the best physical reference as an introduction to ant morphology.

Head

1. *Antennal scrobes*. F/FL: (0) extending above, well behind, and underneath eyes; (1) vary in shape but never surrounding the eye's area. State 0 is present only in *Paraponera clavata* and so far seems to be an autapomorfic feature related to this genus (Bolton 2003). Palacio and Fernández (2003); Arias-Penna (2007).

2. *Torulus*. F/FL: (0) completely fused to frontal lobe; (1) incompletely fused to frontal lobe, joining it at the anterior-mid dorsal portion; (2) distinctly separated from frontal lobe in all its extension. The "complete fusion" character state is easier to interpret when frontal lobes are present as for example in *Pachycondyla*. In *Cephalotes*, however, this state may result much more difficult to identify due the extreme development of the frontal carinae in this genus. Palacio and Fernández (2003); Bolton (2003).

3. *Frontal lobes*. F/FL: (0) absent; (1) present. Here, the frontal lobes are defined as the lateral expansions of the frontal carinae that cover partly or completely the antennal sockets.

4. *Outer borders of frontal* lobes. F/FL: (0) absent; (1) forming short triangle-like structures which are clearly visible for example, in *Pachycondyla*; (2) in the form of rectangular, ovoidal, or semicircular-like structures. In *Camponotus,* unless careful observation, this state can be difficult to visualize and interpret. Modified from Bolton (2003).

5. *Position of head related to the horizontal body axis*. F/P: (0) hypognathous; (1) prognathous. According to Baroni-Urbani *et al.* (1992) and Bolton (2003) the head of ants is turned upward so that mouthparts are directed forward (state 1). A hypognathous oriented head (state 0), instead occurs in most Hymenoptera when mouthparts are directed downward. Modified from Baroni Urbani (1992); Bolton (2003).

6. *Fronto-clypeal sulcus*. F: (0) not reaching antennal sockets; (1) reaching antennal sockets and extending at most to their posterior margin; (2) extending beyond the posterior margin of the antennal sockets. State 0 is shown in *Camponotus*, this is one of the main features that characterizes the genus. State 1 is found in the genera *Ectatomma* and *Dolichoderus*, and finally state 2 is depicted by the species in Ponerinae, *like Pachycondyla carinulata*. Modified from Keller (2011).

7. *When horizontal fronto-clypeal sulcus reaches antennal sockets**. F: (0) suture is clearly visible and usually a triangular-like or oblong-like supraclypeal area is present; (1) suture is not as evident as in state 0, there is a continuum of rugosities and striae extending from the clypeus to the supraclypeal area and beyond; (2) suture is poorly visible; (3) suture is vestigial or strongly faint. State 2 in this study is characteristic of *Cephalotes*, whose frontoclypeal suture is depicted as a very thin line which is better distinguishible from a fronto-lateral view. *Platythyrea* is characterized by state 3 due to the almost completely lack of this epistomal line between the clypeus and frons. However, with careful observation and moving the specimen against different directions of light, it is possible to observe some degree of differentiation.

8. *Supra clypeal area*. F: (0) not differentiated; (1) smooth or slightly ridged and usually with a triagular-like form; (2) longitudinally oblong and relatively slender; (3) triangular shaped with both of its anterior angles projecting anteriorly, composed of fine marginal striae and a smooth core; (4) with no apparent shape but composed of few evident longitudinal protruding ridges. In the genus *Cephalotes* the the supra clypeal area is not visible though in *C. basalis* is vestigial (state 0). A Triangular like-form (state 1) is represented in *Camponotus* but also in *Dolichoderus*. States 3 and 4 are possibly autapomorphies of the genus *Paraponera* and the species *Ectatomma* tuberculatum. Modified from Hölldobler and Wilson (1990), Wilson (2003), Keller (2011).

9. *Frontal carinae*. F: (0) small, extending at most to the external lateral area of the antennal sockets; (1) highly developed, extending laterally far beyond the antennal sockets such that they are comletely covered along with the genae. State 1 is probably an autapomorphic character of *Cephalotes,* such extreme carinal extensions are one of the best synapomorphic traits present in the genus and probably are the

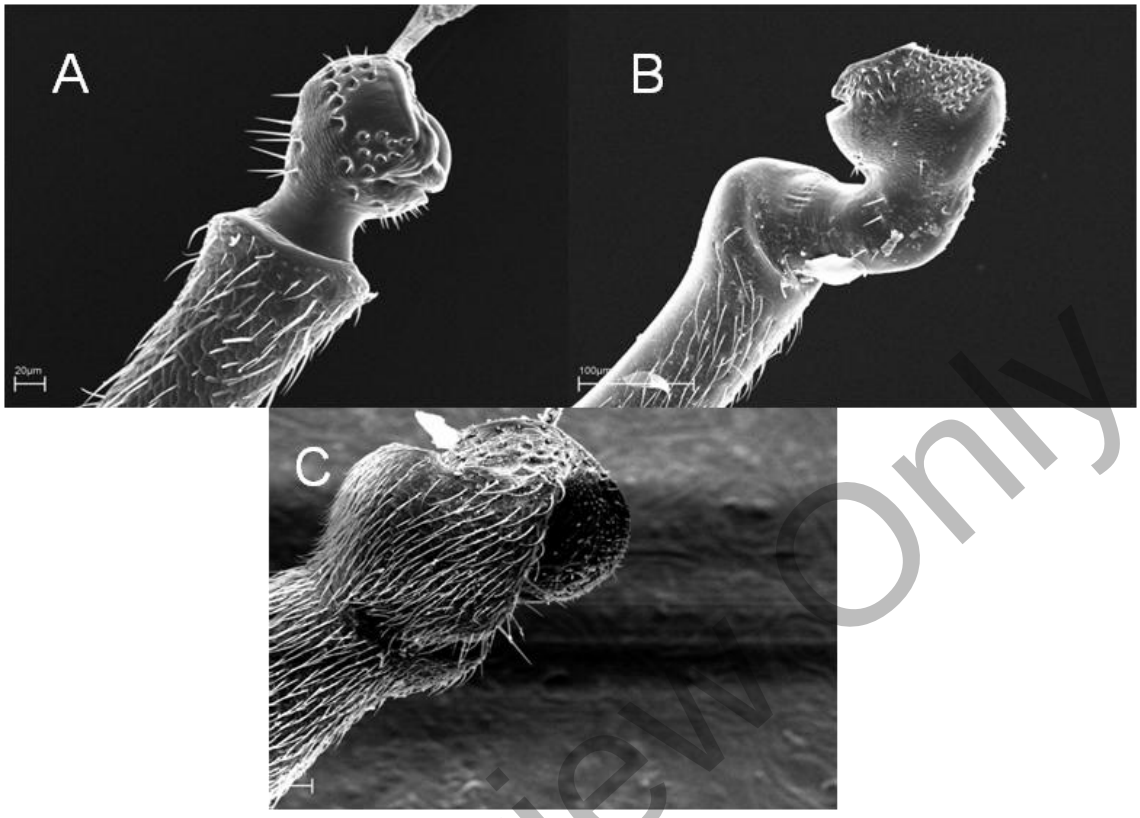
best suitable body features for identifying cephalotes workers. Modified from de Andrade & Braroni Urbani (1999).

10. *Location of carina-frontal lobe fusion*. FL/L: (0) frontal lobe is separated from carina; (1) fusion is discernible only in the anterior portion, close to the torulus median arch; (2) fusion is visible along both structures, but a broad vestigial line of separation is present (see details below); (3) fusion of both structures is complete, there are no vestigial joints. State 1 characterizes *Odontomachus*, in the torular median arch area approaching backguards to the posterior face of the frontal lobe, there is a region where both the frontal lobe and torulus are connected by a broad cuticle which suggests a possible vestigial separation. Modified from Keller (2011).

11. *Frontal carinae posteriorly extending**. FL:(0) maximum to one third of the eye's anterior margin; (1) overpassing the eye's middle region and extending at most to its posterior margin; (2) beyond the eye's posterior margin. State 0 is represented in the genera *Ectatomma* and *Camponotus*. State 1 is shared here by *Dolichoderus* and the ponerine ants, while State 2 is represented only in *Paraponera clavata* and forms part of the scrobal structure which is currently considered a unique derived trait of this species (Bolton 2003). This character is considered here an interesting source for species boundaries especially among Myrmicinae and Ponerinae.

12. *Frontal cariane laterally extending**. F: (0) to no more than the outer lateral border of the antennal sockets; (1) beyond the lateral border of antennal sockets and dorsally covering part of the eyes. Lateral extension of frontal carinae, beyond the eyes, is represented here only by species in *Cephalotes* and by the ponerine genus *Platythyrea*.

13. *Antero-lateral region of torulus*. F: (0) shallow enough allowing a complete visibility of the bulbus at the torular articulation; (1) torular walls present so that the bulbus is partially concealled. In state 0, usually in *Camponotus*, fronto-lateral torulus ridges are not only small enough to permit a broad bulbus visibility, but also are relatively wider than in other genera of this study, e.g. *Dolichoderus*, such that the bulbus muscles are quite visible too. Modified from Keller (2011).



SEM 1. *Bulbus-neck junction*. A. *Dolichoderus superaculus*; B. *Odontomachus hastatus*; C. *Platythyrea angusta*.

14. *Bulbus-neck junction**. F: (0) does not form an angle being the cilindrical bulbus neck clearly visible; (1) elbowed (geniculate) so that a relative right angle is formed, the bulbus neck is clearly visible; (2) reduced so that the apex of the triangular-shaped bulbus neck slighly overlaps the external condylar area of the bulbus. State 0 (SEM 1 A) is distinguishable here in all the genera (except *Ectatomma*) of the formicoid clade, e.g., *Dolichoderus* (Appendix 2: L, M, N). State 1 (SEM 1B) is represented here by the species comprising the poneroid clade, e.g. *Odontomachus* (Appendix 2P). State 2 (SEM 1 C) characterizes *Platythyrea*, its bulbus is unique among the set of species in this study (Appendix 2V). In small species like *Cephalotes maculatus* enhanced visibility of this character is obtained by extracting the whole structure.

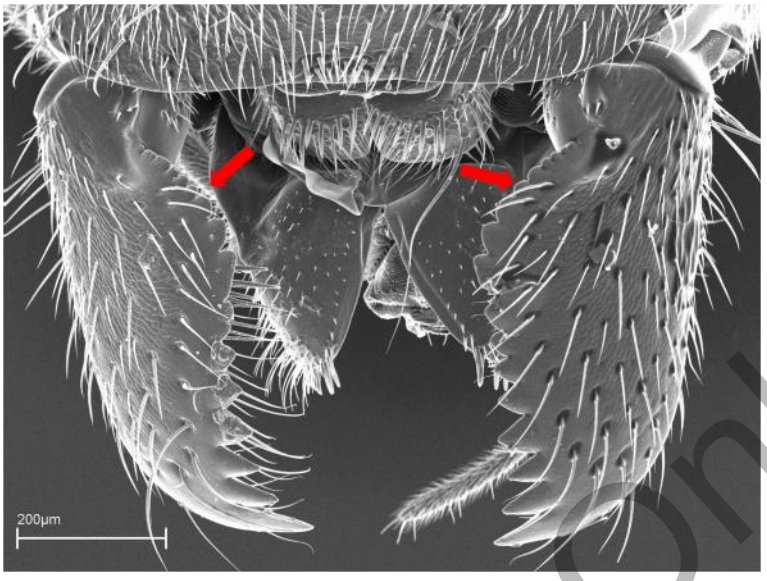
15. *Size of basal margin of mandible related to the masticatory margin**. F/FL: (0) slightly shorter, so that masticatory and external margins together are triangular shaped; (1) much shorter, thus mandibles are comparatively very slender and long;

(2) about the same length or slighlty longer, so that masticatory and external margins form a rectangular-like shape; (3) much longer than masticatory margin. Triangular-like mandibles (state 0) are more common than the other states shown here, these mandibular forms are represented usually in predatory ants, e.g., *Pachycondyla inversa*, but also are quite common in omnivorous ants, e.g., *Camponotus* spp. State 1 is unusual in ants and suggestive of specialized predatory behavior (Brown and Wilson 1959, Hölldobler and Wilson 1990), in this study only *Odontomachus* shows it. Rectangular shaped mandibles (state 2) are less common in ants, here are present in all *Cephalotes* species and in several *Camponotus* species.

16. *Shape of basal margin of mandible**. F: (0) smooth and simple with no tooth or denticles; (1) with one triangular shaped tooth located close to its inner border; (2) with several denticles along its ridge, decreasing in size as they approach to the inner most border. State 0 is present in most of the ants analyzed here, in contrast, state 1 is only present in *Paraponera* clavata, and finally state 2 is present in all dolichoderine species (genus *Dolichoderus*) (SEM 2). The function of the tooth in *Paraponera* and denticles *Dolichoderus* species is unknown, but possibly they aid in ripping up their prey tissues and/or grabbing nest materials.

17. *Accessory chamber of antennal sockets.* F: (0) absent; (1) present. These accessory chambers of unknown function are composed of a blind cuticular vesicular attached internally to root of the cranium (Keller (2011). Possibly is not a common structure in ants. In this study here is represented by *Ectatomma* species.

18. *Anterior most clypeal carina.* FL: (0) absent, this is, there is not ridge or shelf-like border at the anterior most clypeal region; (1) present. This character state does not vary dramatically in ants as does for example the clypeal area, shown in works of Keller (2011), Brady and Ward (2005), this study is not the exception, however here it is coded as it represents another morphological split between *Pachycondyla* (state 1) and *Odontomachus* (state 0). Modified from Keller (2011).

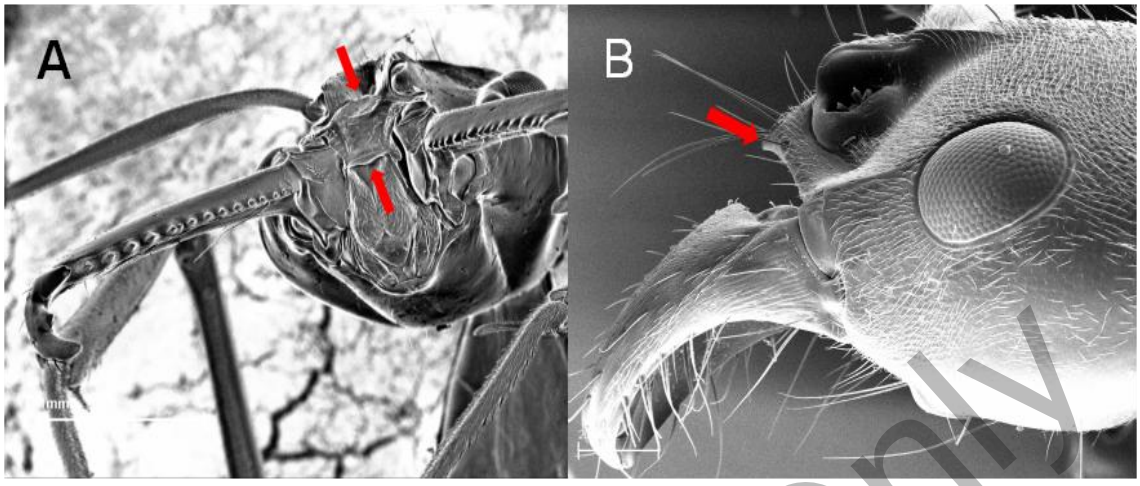


SEM 2. *Shape of basal margin of mandible in Dolichoderus* (shown by the arrows).

19. *Clypeo-labral articulation*. FL: (0) concealed by overhanging clypeus; (1) exposed. The former state is represented in most of species across Formicidae, e.g. *Platythyrea angusta*, or *Pachycondyla carinulata* (SEM 3B) in this study (Appendix 2V), and possibly also in the Aculeate clade, in contrast, an exposed clypeo -labral articulation is rare. According to Ogata (1991) and Ward and Brady (2003) this latter state is autapomorphic in *Myrmecia*, however here I show here that it is also present in *Odontomachus* (SEM 3A). (Appendix 2P) Ward and Brady (2003).

20. *Compound eyes*. FL: (0) polished and brilliant, apparently without facets; (1) different than state 0. In this study polished eyes are a unique characteristic of the outgroup *Dasymutila occidentalis*. Modified from Keller (2011) and War and Brady (2003)

21. *Size of eyes**. FL: (0) large, notched, and ocupaying more than half the total length of the head; (1) smaller than in state 0, at most ocupying 1/3 of the total length of the head. Very large eyes (state 0) and usually notched are notably present in most of the members of the Aculeate clade.



SEM 3. *Anterior most clypeal carina* and *clypeo-labral articulation*. A. *Odontomachus hastatus* mouthparts: top red arrow indicates the lack of a frontal carina; bottom arrow, an exposed clypeo-labral articulation. B. *Pachycondyla carinulata*, showing the frontal carinae typical of this genus and in many ponerines.

22. *Distance between the eye's anterior border and the lateral most region of the clypeo-genal suture, close to the mandibular articulation*. FL: (0) smaller than the eye's greater diameter; (1) larger that the eye's greater diameter. Modified from Ward and Brady (2003).

23. *Antennae*. FL: (0) nongeniculate; (1) geniculate between scape and funiculus. Geniculate antennae occurs in all ants and in the Vespidae, although in most species of the latter, the first antennal segment (scape) is relatively reduced compared to the antennal scape in most ant species. First antennal segment of Mutillidae in this study is similar to a "true"scape", the difference is that mutillid scapes are curved outwards as seen dorsally, but also no elbowed-like (geniculate) articulation is formed. Modified from Bolton (2003).

24. *Anterior border of clypeus*. D/FL: (0) straight or slightly convex; (1) concave or cleft; (2) acute, so that a blunt prominence is clearly visible in its tip. An acuminate clypeus is usually present in *Pachycondyla* species but also in other ponerines, e.g. *Leptogenys*; in this study *Ectatomma* exemplars also show it. Concave clypeus (state 2) is shown in *Cephalotes* but also in one species of *Dolichoderus*. All the species in *Camponotus* are placed under state 0. Modified from Ward and Brady (2003) .

25. *Distal lateral portion of the clypeus at the clypeo-genal suture**. D: (0) acuminate forming a triangular-shaped area, usually with its vertex slighlty directed frontally, an oblique suture line marks the boundaries between the clypeus and the gena; (1) not acuminate, instead it is rounded or square-like. State 0 is in this study is represented by *Camponotus* species.

26. *Abundant erect hairs covering the pleurostomal margin**. P: (0) absent; (1) present. In this study *Paraponera clavata* exemplars show state 1, although pleurostomal hairs are present in three species of *Pachycondyla* considered here, but these are not so abundant and they are just present in the inferior part of the pleurostomal margin.

27. *Hairy pleurostomal margin (extension of char. 26)* *. P: (0) with relatively short standing setae (~0.08 mm in average) and less abundant in the region in front of the mandibular insertion; (1) standing setae are longer than in previous state (~0.19 mm average), and evidently more abundant. A hariy pleurostomal margin is apparently ubiquitous of *Paraponera clavata* here, and there is a clear morphological difference between exemplars from Chocó and Amazon regions. Specimens of *P. clavata* from Chocó represent state 1.

28. *Preocular carina.* DL: (0) absent; (1) present. The latter state is usually present in many Neotropical species of *Pachycondyla* (SEM 3B), so far this has been attested in all exemplars collected in Ecuador (per. obs.). Although the preocular carina has long been considered as a "good" character to arrange the phylogenetic relationships within this heterogeneous genus, currently it lacks taxonomic weight due to its plasticity. Mackay and Mackay (2010)

29. *Median longitudinal clypeal carina (antero-posterior body axis).* F: (0) completely absent; (1) partially absent, the carina is not easily visible and not well formed as a "true" ridged cuticular evagination; (2) present, running usually from the anterior to the posterior clypeal border. An absence of this structure (state 0) is seen e.g. in *Pachycondyla* and *Cephalotes*. Is partially absent (state 1) e.g. in *Camponotus sericeiventris*, and is evident (state 2), e.g. in *Ectatomma* and other *Camponotus* like C. sp. 6. Modified from Keller (2011)

30. *Additional variation of state 1 in char. 29 (clypeal carina)**. F: (0) a faint and poorly appreciable line is present, usually is required to move the specimen in several directions against light to visualize this structure; (1) the structure is better appreciable than in state 0 and is not required to manipulate the specimen against light. Variations of this character are recognizable only in species of *Camponotus*. Sate 0 is depicted e.g. by *Camponotus* sp. 5, whereas state 1 can be recognized in *C. sericeiventris*.

31. *First segment of funiculus (part i)*. *P*: (0) smaller than the second; (1) around the same size compared to the second; (2) longer than the second. Following Bolton (1994, 2003) the funiculus is the part of the antenna comprising all the antennomeres with the exception of the scape (1st antennal segment). State 0 is depicted in e.g. *Paraponera clavata*, while state 1 is present in all the species of *Pachycondyla* , e.g. *P. carinulata* (Appendix 2Q), and finally state 2 is present in all species of *Camponotus* and in most species of *Dolichoderus* in this study, e.g. *Dolichoderus* sp.2. (Appendix 2: L,M,N), Modified from Ward and Brady (2003); Brady and Ward (2005).

32. *First segment of funiculus (part ii)*. P: (0) much smaller than the second, at most 1/3 or less the size of the second segment; (1) smaller, 1/2 the size of the succeeding segment. State 0 is common in many species within Hymenoptera, here is represented by the outgroups. State 1 is shown in *Odontomachus hastatus* and *Paraponera clavata*. Modified from Ward and Brady (2003).

33. *Antennal scape*. P: (0) short, at least 1/3 the size of the funiculus; (1) long, around the same size of the funiculus. A relatively long scape similar to that present in many Vespidae for example, is a characteristic feature of female Formicids, so far recognized for all known species. Modified from Ward and Brady (2003).

34. *Standing conspicuous hairs across the antennal scape area**. F/D: (0) absent; (1) present. Amount and position of standing hairs in ant antennal scapes varies considerably among species, thus making this character homoplastic, however in this study I found it useful to differenciate the clades analyzed within Ponerinae: *Phachycondyla* shows state 1, while *Odontomachus* and *Platythyrea* show state 2.

35. *Median longitudinal cephalic carina*. F: (0) absent; (1) present. When present, the cephalic dorsum bears a strong longitudinal carina which may run from the anterior part of the clypeus or from the supraclypeal area to the vertex (posterior border of the head) (Brown 1958, Lattke 1994, Keller 2011). In this study, a very clear median cephalic carina is only present in *Ectatomma*. Variations of this cuticular structure are found is many species within the subfamilies Ectatomminae and Heteroponerinae. Nonetheless I found similar cuticular projections and impressions in all the species of *Camponotus* analyzed in this study, however these are less evident/protruding that those present in the mentioned subfamilies, and do not extend to the vertex. A separate character for this variation is defined below (char. 36).

36. *Impressed cuticular line between antennal sockets*. F: (0) absent; (1) poorly visible and not protruding; (2) clearly visible but not protruding; (3) clearly visible and protruding. State 1 can be very difficult to identify as in e.g. *Camponotus sericeiventris*, however moving the specimen in different light-oriented positions enhances the visibility. State 2 is shown in e.g. *Camponotus* sp. 4. Finally state 3 is represented only in *Camponotus* sp. 6, and *Camponotus* sp. 7 from Chocó. Modified from Keller (2011).

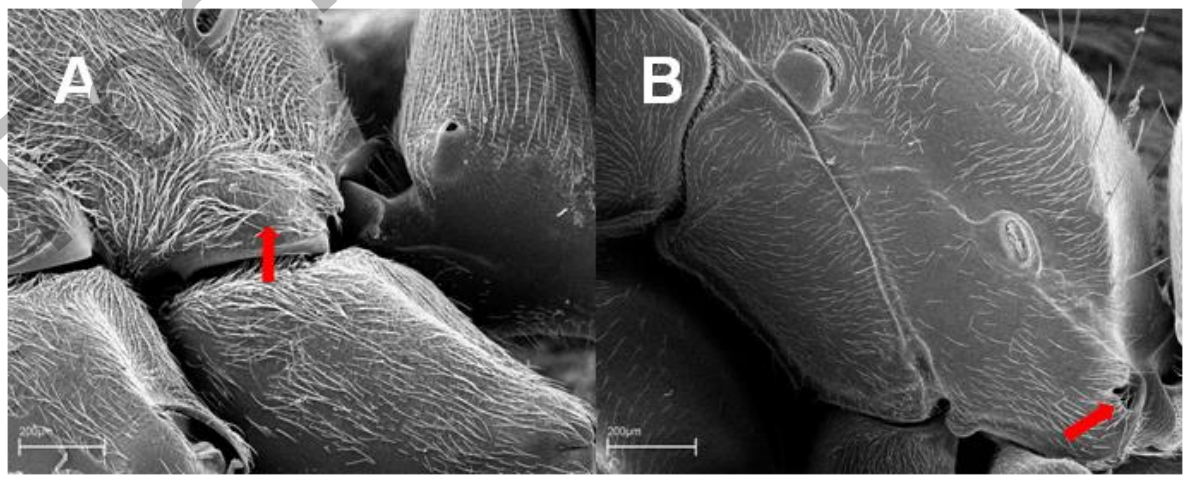
37. *Number of maxillary palps.* V: (0) 6; (1) 5; (2) 4; (3) 2. Maxillary palps in the Formicidae can vary from one to six, being the latter the most common. Species in *Camponotus*, *Dolichoderus* and *Platythyrea* show six maxillary palps (state 0). Five palps (state 1) are shown in *Paraponera clavata* and *Cephalotes*. Four palp joints (state 2) are present in all the ponerines excepting *Platythyrea*. Finally, two palps (state 3) are represented by *Extatomma*. (Keller (2011); Lattke (1994).

38. *Number of labial palps*. V: (0) 4; (1) 3; (2) 2. In ants labial palps vary from four to none, being the former the most common. Four labial palps (state 0) were found in most of the ants in this study, for example in *Caponotus*, or in the ponerines excepting *Odontomachus*. Three labial palps (state 1) are represented in *Paraponera clavata* and *Cephalotes* species. Finally, two labial palps are present only in the genus *Ectatomma*. Lattke (1994); Keller (2011).

39. *Sensorial hairs located in the last maxillary palp*.* V: (0) absent or minuscule such that they represent less than 1/5 the length of the palpus; (1) two or more present but they are around 1/3 the length of the palpus; (2) with two or more very clear and long hairs, in average their length is close to half that of the palpus, or longer. Terminal sensorial hairs probably aid in food search and nestmate recognition. State 0 is absent in all *Camponotus* species in this study. State 1 is present only in *Dolichoderus*, and state 2 is depicted by species in the genus *Pachycondyla*.

40. *Distance from the eye's posterior margin to the vertex**. F/P: (0) less than the eye's diameter; (1) around the same length of the eye's diameter or longer. In some species in this study the eyes are located close to the vertex of head (state 0), this is the case of some *Camponotus,* like *Camponotus* sp. 4, or species in *Cephalotes*. The opposite arrangement (state 1) is depicted by most species in this study, e.g. *Pachycondyla inversa*.

41. *Spheric bulging eyes**. F: (0) absent; (1) present. This character refers to species in which the eyes are clearly protruding from the head's surface, this is, more than half of the eye's sphere is emergent out of the head's cuticle, as in some species of *Camponotus*. The other case coded in this study (state 1) is represented in most ant species, e.g. *Dolichoderus lugens,* where a relatively small proportion of the eye's sphere emerges from the head's cuticle. Modified from Mackay (2007).



SEM 4. *Metapleural gland (MG)*. A. *Camponotus sericeventris* without MG, the arrow shows the place where it should be located close to the vertex of the metapleuron. B. *Pachycondyla carinulata* showing an evident MG orifice.

Mesosoma

42. *Prepectus*. P: (0) absent; (1) present. The postspiracular sclerite also called prepectus is a character present across the Hymenoptera, its absence is suggestive of a primitive state among the "Symphyta", group that comprises several basal hymenopteran species. Conversely, this structure is present in the rest of the Hymenoptera (Gauld and Hanson 1995).

43. *Metapleural gland*. P/DL: (0) absent; (1) present. The metapleural gland is one of the best synapomorphic characters among the Formicidae, but is also a unique glandular structutre so far only known in ants, there are exceptions though. For example in several *Camponotus* is absent as there is no orifice at the vertex of the metapleuron, where it should be located. Instead, a small round bulge is visible in this sclerite (SEM 4). Hölldobler and Engel-Siegel (1984); Palacio and Fernández (2003).

44. *Ventral flap on metapleural gland opening*. P/DL: (0) without metapleural gland; (1) absent; (2) bulla shaped. When the latter state is present the orifice of the metapleural gland forms a slit-like curved narrow openning directed dorsally to posterodorsally. An absence of a flap over the metapleural gland is shown for example in *Dolichoderus* here. When this structure has a globular shape, resembling a “bulla”, the metapleural gland opening is hard to observe as it is camouflaged by that structure. This state is represented only in *Ectatomma* in this study. Modified from Bolton (2003) and Keller (2011).

45. *Dorsal promesonotal junction*. D: (0) articulated; (1) sutured and not movile; (2) fused and almost indistinct. The promesonotal junction is defined as the point of confluence of the posterodorsal margin of the pronotum and the anterior margin of the mesonotum. It is articulated (state 0) e.g. in all *Camponotus* and *Dolichoderus* here (Appendix 2: A - I). This structure can also have a vestigial suture (state 1, as in species of *Pachycondyla*) remainder of past motility. Finally, it can be fused but also hard to distinguish (state 2, as in *Cephalotes*). Modified from Keller (2011).

46. *Color of mesosoma**. D/P/V: (0) light brown; (1) redish black; (2) dark brown; (3) black. Unlike many insect families, ants are not colorful, in general it varies from black to brown, to redish (including their variations), in most cases. Slight variations (e.g. from brown to light brown) can be present even in individuals of the same colony. However, these are omitted here to avoid bias in the analyses. A light brown color (state 0) is shown e.g. in *Camponotus* sp. 2 (Appendix 2: A - I). A redish color (state 1) is depicted by the Amazonian *Paraponera clavata* (Appendix 2: U2). . A dark brown mesosoma (state 2) is shown in *Odontomachus hastatus* from Chocó (Appendix 2: P1). Finally, a black mesosoma is the dominant variant, e.g. in *Dolichoderus* sp. 2 (Appendix 2: L - N).

47. *Meso-metapleural suture*. P: (0) absent; (1) present. This suture referes to the junction between the mesopleuron (anepisternum + katepisternum) and the metapleura. The suture is absent in some *Camponotus* species like *Camponotus* sp. 8 (Appendix 2I) in this study. Whereas it is present in the majority of the species here analyzed, like *Pachycondyla carinulata* (Appendix 2Q). Modified in part from Keller (2011).

48. *Notopleural suture on mesothorax*. P: (0) absent; (1) present. This structure is defined here as the place of junction between the mesonotal plate and the mesopleuron, it is absent (state 0) in many species here, e.g in *Camponotus* sp. 8 (Appendix 2I). The suture is present in all the ponerine ants analysed here like e.g. *Pachyconyla carinulata* (Appendix 2Q). Keller (2011).

49. *Dorso-lateral emergent pronotal prominences*. DL: (0) absent; (1) present in the form of spines; (2) present in the form of acumminate or dull lobes but different from real spines; (3) present in the form of lamellae. Pronotal spines and prominences are not a common feature among the species of the present data set. Spines (state 1) are found e.g. in *Dolichoderus superaculus* (Appendix L1) and (SEM 5A), while acuminated dull lobes (state 2) are shown in *Ectatomma tuberculatum*, *Paraponera clavata* (Appendix 2U) or *Dolichoderus* sp. 3 (SEM 5B). Pronotal lamellae (state 2), defined here as lateral cuticular extensions in the form of plates (e.g. *Cephalotes,* Appendix 2: J, K) are the less common in this study, in fact it is only known to occur in

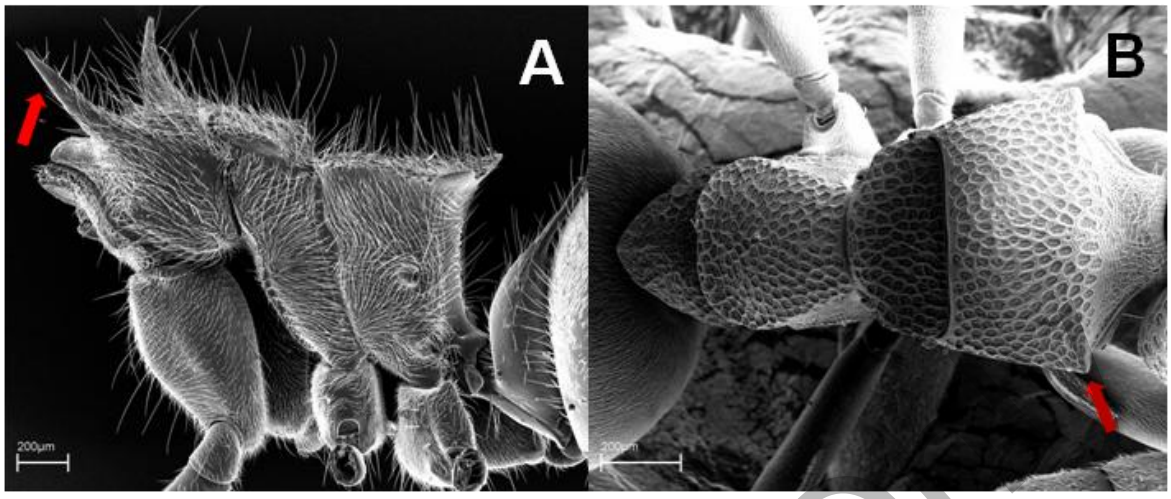
two clades, Cephalotini and Cataulacini, among the Formicidae. Modified from Keller (2011).

50. *Pronoto-mesepisternal junction*. P: (0) articulated; (1) sutured. The joint between these two structures is located at the antero-dorsal part of the mesosoma. The former state is more common among specimens of the present data set, which is depicted e.g. in *Pachycondyla carinulata* (Appendix 2Q). A sutured joint (state 1) is shown in *Paraponera clavata* (Appendix 2U). Keller (2011).

51. *Metanotum either.* D: (0) not visible dorsally; or (1) clearly visible dorsally. The metanotum of all the ant exemplars considered is this study is not visible dorsally (state 0) as it is completely fusioned either, with the mesonotum by its frontal part or with the propodeum by its posterior part. In contrast, the metanotum is clearly visible in other vespoidea like the Mutilids examined here (as outgroup), and also is plenty visible dorsally in most of the basal Hymenoptera, like the argids (Argidae) also examined here as outgroup. Modified from Keller (2011).

52. *Dorsal suture between meso-metanotum and propodeum*. D: (0) absent, such that both structures are completely fused; (1) present as a faint or clear line, but in any case both structures are not separated; (2) present in the form of a relatively deep channel that separates the propodeum from the mesometanotum. The suture is absent (state 0) in *Odontomachus hastatus*. In *Camponotus sericeiventris* this suture (state 1) may be very difficult to visualize as the mesosoma is fully covered with abundant hairs. State 2 is depicted here by species in *Dolichoderus*, e.g. *Dolichoderus* sp. 2 (Appendix 2: L- N). Modified from Keller (2011).

53. *Metathoracic spiracle*. DL: (0) concealed by a spiracular lobe; (1) exposed with the opening being round to oval. A concealed spiracle (state 0) is found here for example in the ponerine ant *Pachycondyla carinulata* (Appendix 2Q). In contrast an exposed spiracle (state 1) is more common, e.g. in formicine ants like *Camponotus sericeiventris* (Appendix 2 A). Modified from Keller (2011).



SEM 5. *Dorso-lateral emergent pronotal prominences*. A. *Dolichoderus superaculus* showing prominent pronotal spines. B. Doichoderus sp. 3 with blunt pronotal prominences different from "true" spines.

54. *Metathoracic spiracle orientation*. D/DL: (0) dorsolateral; (1) posterolateral. The former state is shown in all the formicines and dolichoderines here, e.g. *Camponotus sericeiventris* (Appendix 2A), while state 1 is more common in the ponerines, e.g. *Pachycondyla carinulata* (Appendix 2Q). Keller (2011).

55. *Opening orientation of metapleural gland*. P: (0) metapleural gland absent; (1) lateral; (2) postero-dorsal; (3) posterior. When present, a lateral opening of the metapleural gland is featured in *Cephalotes* or *Paraponera clavata* in this study. State 2 is characteristic of the ponerines here like e.g. *Pachycondyla carinulata* (SEM 4B). Finally a posterior opening is found here in all dolichoderines. Modified from Keller (2011).

56. *Propodeal spiracle atrial opening*. D/P: (0) slit shaped; (1) round to oval. In the former state the spiracle opening is very slender longitudinally, much thinner than wide (SEM 4B) e.g. all the ponerines except *Odontomachus*. On the other side it can be shaped as an oval or round aperture (state 1) as in most of the formicines, e.g. *Camponotus* or *Dolichocerus* (SEM 5A). Keller (2011).

57. *Propodeal lobes*. P/DL: (0) absent; (1) present. The propodeal lobes are cuticular extensions of the propodeum present mainly in its posterior part. These can be

oriented in several directions and, according to Keller (2011), sometimes confused with metapleural gland extensions (e.g. Brady and Ward 2005) or misidentified as inexistent (e.g. Bolton 1994; Brandão *et al.* 1999, Bolton 2003).

58. *Mesosomal dorsal carina**. D: (0) absent; (1) present. The dorsal carina in this case corresponds to an evident projection of the msesoma originating at the pronoto-metanotal dorsal junction and extending posteriorly to the dorsal edge of the propodeum. In this study, this character is present only in *Camponotus sericeiventris* (Appendix 2A) and probably in an autapomorphy of this genus among the Formicidae.

59. *Width of the dorso-mesosomal carinal edge**. D; (0) mesosomal dorsal carina absent; (1) do not vary across its entire length; (2) becomes wider starting on the mid propodeal area, having double the width of its anterior part when it reaches the vertex of propodeum. State 1 is represented by *Camponotus sericeiventris* from Chocó region, while state 2 is present in *C. sericeiventris* from Amazonia.

60. *Sutural line dividing anepisternum from katepisternum*. P: (0) absent; (1) present, although faint and poorly visible; (2) present and clearly visible. In state 1 this line can be confounded with adjacent striae covering the surface of the mesopleuron (anepisternum + katepisternum), this case is represented here by *Ectatomma*. Modified from Keller (2011).

61. *Standing hairs in the dorsum of the body*. P/D: (0) absent; (1) showing few dozens with broad easily discernible gaps between them; (2) several dozens present with few gaps in between. Keller (2011).

62. *Form of the posterior face of propodeum**. P: (0) convex, so that it forms with the dorsal face a round border; (1) straight or with a slight concavity, so that it forms with the dorsal face an acute or straight angle. State 0 is normally present in most of the species in the family, whereas state 1 is not as usual, here is depicted only by *Dolichoderus* species.

63. *Dorso-posterior carina located at propodeal vertex**. P: (0) absent; (1) present. In ants, this structure of the propodeum is relatively rare (*pers. obs*.). Here is shown in dolichoderine species and in *Ectatomma tuberculatum.*

64. *Orientation of carina in dorso-posterior vertex of propodeum**. P: (0) carina absent; (1) upwards; (2) oblique, tilted backwards. A propodeal carina projecting upwards (state 0) is present only in *Dolichoderus lugens* in this study, while an oblique carina (state 1) is represented for example in *Dolichoderus superaculus*.

65. *Cuticular shape of metapleuron**. P: (0) smooth with no rugosities o foveae; (1) mainly striated or with rugosities; (2) mainly with foveae of different size.

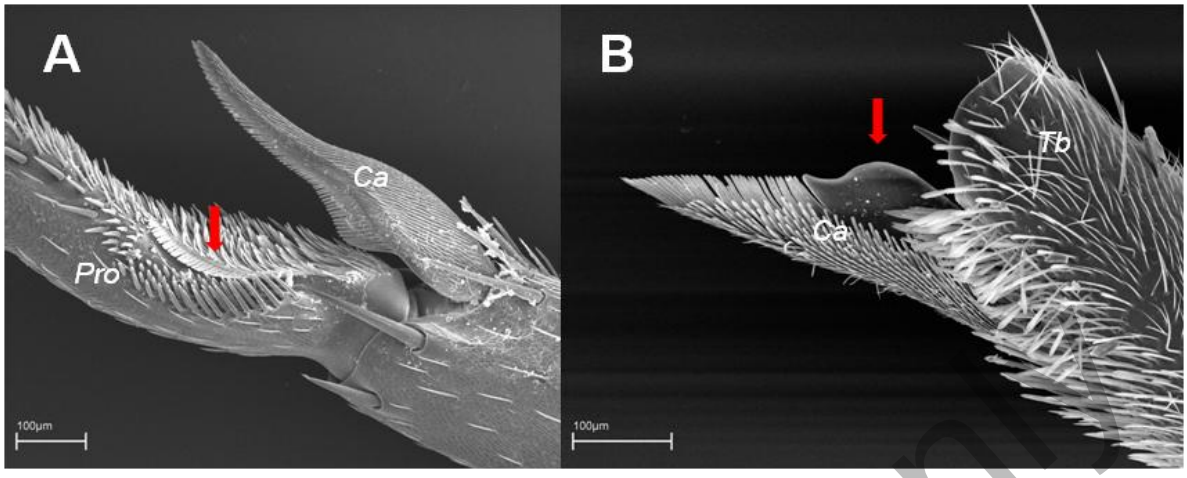
Legs

66. *Spine-like stout setae on tibial spurs**. L/V: (0) absent; (1) present. State 1 is present in *Dasymutilla occidentalis*, as well as in many species belonging to this genus. These stout and short setae, arranged in two longitudinal rows in each tibial spur, provide at first sight an appearance of a saw-shaped strucutre.

67. *Metatibial spur*. P/V: (0) absent; (1) present. Normally, the metatibia bears two spurs, an anterior spur and a more posterior one, being the former smaller and less complex than the posterior one (Keller 2011). Here I have focused on the posterior metatibial spur. This spur is present in all the exemplars observed with the exception of *Cephalotes* species. Keller (2011); Ward and Brady (2003); Brady and Ward (2005).

68. *Pectinated metatibial spur*. P/V:(0) absent; (1) present. Keller (2011); Ward and Brady (2003); Brady and Ward (2005)

69. *Size of modified setae (pectinae) in metatibial spur**. P/V: (0) pectinae absent in metatibial spur; (1) tiny, the length of the setae are less than half the tibial width; (2) longer than in state 1, length of setae is clearly longer than the tibial width.



SEM 6. *Antennal-cleaning structure (strigil) in the probasitarus*. A. *Camponotus sericeiventris* showing a pectinated calcar. The arrow shows the probasitarsal notch. B. *Pachycondyla carinulata* with a composite calcar: pectinae + *vellum* (= lamella). The arrow indicates the *vellum* showing a tiny apical notch. Abbreviations: *Pro*, probasitarsus; *Ca*, calcar; *Tb*, Tibia.

70. *Calcar of foretibial spur*. P/V: (0) fully pectinated; (1) with a basal lamella or *vellum* (Schönitzer and Lawitzky, 1987) and pectinae in the apical part; (2) fully lamellated. Modified from Brady and Ward (2005), and Keller (2011). State 0 is represented for example in *Camponotus sericeiventris* (SEM 6A), while state 1 is depicted in all the ponerines here, e.g. *Pachycondyla carinulata* (SEM 6B). State 2 is represented by the outgroup *Dasymuntilla occidentalis*.

71. Lamella of calcar: P/N: (0) calcar without lamella; (1) entire; (2) with a small almost imperceptible notch; (3) with a clear evident notch. An entire lamella, this is, without any notches or depressions along its length (state 1) is found in the outgroup *Dasymutilla occidentalis*. A tiny lamellar notch (state 2) discernible with maginifications above 50X (depending on the size of the specimen) is shown for example in *Pachycondyla carinulata* (Appendix 2Q) (SEM 6B). Finally, an ubiquitous concavity much perceptible than in state 2, is found in *Odontomachus* exemplars analyzed here. State 1, as coded in this study, departs from the observations of Keller (2011:46) who defined an "entire lamella" for *Pachycondyla* species. Present observations on *Pachycondyla* specimens (e.g. *P. crassinoda*) that Keller (2011) analysed in his work do not show such "entire calcar lamella" being otherwise equal to state 2 coded in this study. Modified from Keller (2011)

72. *Brush on posterior surface of calcar*. P/V: (0) absent; (1) present. Keller (2011)

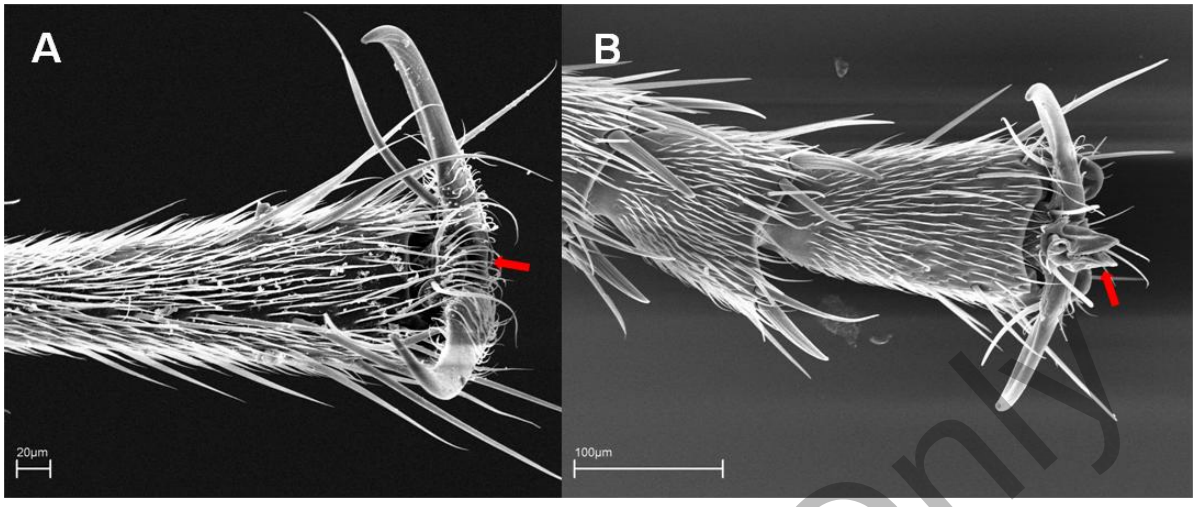
73. *Spine-like setae in the outer part of probasitarsal notch*. P/V: (0) absent; (1) present. In ants, basitarsal notches (SEM 6A) are located usually in the upper region of the probasitarsi. There are several sets of hairs and spines around this area, in this study a set of five or more spine-like setae located in the middle area of the probasitarsal notch have been recognized. These are absent for example in *Platythyrea* species, but are present in most of the exemplars examined here, e.g. in all species of *Camponotus.* Modified from Keller (2011).

74. *Probasitarsal notch*. P: (0) absent; (1) present. The probasitarsal notch is a ventrobasal emargination located in almost all ants (and in most Aculeates) close to the basitarsal apex in which there is a row of modified setae, the basitarsal comb, which, together with the calcar forms the antennal cleaning system (SEM 6A) (Gibson *et al* 1998). This strucutre is quite conserved across Formicidae not showing high inter specific variation probably because of its importance in antennal stimuli reception. Modified from Keller (2011).

75. *Arolium in mesopretarsus and metapretarsus*. V: (0) vestigial to absent; (1) present. The *arolium*, according to Leponce *et al.* (2010) is a pad-like median structure between the pretarsal claws. In arboreal ants this structure is probably highly useful for dwelling across suspended polished leafs up in the canopy branches. A vestigial *arolium* (state 0) is present here in only two species, *Ectatomma tuberculatum* and *Odontomachus hastatus* (Appendix 2P) (SEM 7A). The rest of the species observed here possess aroliae, e.g. *Pachycondyla carinulata* (SEM 7B). Modified from Keller (2011).

76. *No. of claws in pretarsi*. V: (0) two; (1) four. Two claws per tarsus (state 0) is a feature present in most of the exemplars in this study, e.g. *Pachycondyla inversa* or *Camponotus sericeiventris*. Four claws (state 1) are represented in *Platythyrea angusta* for example (Appendix 2: V2). Modified from Keller (2011).

77. *Basal protuberance in pretarsal claw**. V: (0) absent; (1) present as a simple bulge or cuticle thickening; (2) present as a gross bulge resembling a second claw.



SEM 7. *Pretarsus*. A. *Odontomachus hastatus*, the arrow indicates the place of a vestigial *arolia*. B. *Pachycondyla carinulata*, showing the arolia, which is suggestive of arboreal habits.

Metasoma

78. *Second abdominal segment* (the *petiole*). D/P: (0) not differentiated, there is no bridge-like structure or constriction between it and the third abdominal segment; (1) well differentiated usually forming a petiole which shows a constriction between it and the third abdominal segment. State 0 is a derived state possibly present in all "Symphyta" therefore a potentially good synapomorphy. Sate 1 varies greatly among the "Apocrita" (= those Hymenoptera that are not basal clades within the order). The constriction between the second and third abdominal segments is probably one of the best synapomorphies for this group. The petiole in the primitive (fossil) ant subfamily Armaniinae is weekly differentiated from the rest of abdominal segments. Modified from Bolton (2003).

79. *On the petiole and pospetiole*. D/P: (0) only the second abdominal segment isolated or reduced between the mesosoma and gaster; (1) second and third abdominal segments (petiole and postpetiole) are isolated or reduced between the mesosoma and gaster. Palacio and Fernández (2003).

80. *Third abdominal segment*. D/P: (0) entirely confluent with the fourth segment or separated from it by a slight constriction. In the latter case, the first segment of the gaster (in this particular case = the third abdominal segment) is not strongly reduced; (1) as reduced as the second or bigger than it but in both cases notably smaller than the fourth abdominal segment and separated from it by a very clear constriction. Modified from Palacio and Fernández (2003).

81. *Sting*. V/P: (0) absent or vestigial; (1) present. In the dolichoderines this state is vestigial or completely absent, being the former only discernible through dissection. Cuezzo (2003); Palacio and Fernández (2003).

82. *Fourth abdominal segment*. P/V: (0) without tergosternal fusion, both sclerites are motile one respect to the other; (1) with tergosternal fusion, both sclerites are rigidly fusioned and no motile. Palacio and Fernández (2003).

83. *Acidopore at apex of hypopygium*. F/V: (0) absent; (1) present in the form of a tubular projection usually bordered by bristles or covered by the pygidium, this projection is used in formicine ants to secret formic acid through the poison gland. In dolichoderines instead of an acidopore there is a slit-like junction of the pygidium and the hypopygium through which compounds known as "iridoids" are secreted. Bolton (2003); Cuezzo (2003); Palacio and Fernández (2003).

84. *Petiolar sternite.* P/V: (0) articulated with the tergite, there is a relatively clear separation between both sclerites although may be difficult to visualize in small species as for example in *Pachychondyla crenata*; (1) fused with tergite in its entire length showing a clear and thick vestigial line of separation; (2) as in state 1 but showing a very thin faint line between the sclerites. Modified from Keller (2011); Bolton (2003).

85. *Petiolar peduncle*. P/D: (0) sessile or subsessile, it rarely extends more than 1/4 the petiolar length; (1) elongated, usually half the length of the petiole or close to that. Short petiolar peduncles are more common in this study than longer ones. Examples of sessile peduncles are depicted by *Dolichoderus* (e.g. *Dolichoderus* sp. 2, Appendix

2 N), while elongated peduncles are characteristic in *Odontomachus* species (Appendix 2P). Modified from War and Brady (2003); Keller (2011).

86. *Dorsal spine in pedunculate petiole**. P/D: (0) absent; (1) present. The petiolar spine is defined here as a protruding pointed projection emerging dorsally or postero-dorsally from the top of the petiolar dorsal cuticula. The only clear case of a "true" spine in a pedunculated petiole is depicted here in *Odontomachus* species.

87. *Orientation of the external part of petiolar spine in pedunculated petiole**. P: (0) petiolar spine in pedunculated petiole absent; (1) erect, it runs upwards in the same direction as the basal part; (2) curved, it is evident the difference in orientation compared to the basal part. A curved external spine (state 2) as shown in *Odontomachus hastatus* (Appendix 2: P2) exemplar from Amazonia could be difficult to discern depending on the posture of the specimen. An erect position of this structure (state 1) is represented in *O. hastatus* exemplar from the Chocó region (Appendix 2: P1).

88. *Horizontal protruding striae in the petiolar poststernite**. V: (0) absent; (1) present. In many formicid species especially those in the families Ponerinae and Paraponerinae, a series of linear rugosities or striae are commonly distinguishable in the petiolar poststernite as in *Paraponera clavata* for example.

89. *Position of anterior face of the third abdominal segment with respect to the petiole**. P: (0) relatively straight, so that it does not overhang over the postero-dorsal petiolar face; (1) relatively inclined anteriorly, so that it overhangs the postero-dorsal petiolar face. Usually in state 1, the posterior place of insertion of the petiole to the third abdominal segment is not visible dorsally, e.g. *Dolichoderus* sp. 2 (Appendix 2N).

90. *Petiole's length vs height**. P: (0) longer than its height; (1) around the same proportions; (2) higher than its length. For simplicity of state determination, petiolar dorsal extensions (as in *Dollichoderus)*, spines (*Odontomachus*), and lamellae (as in *Cephalotes*) are not considered in these measures.

91. *Orientation of anterior face of petiole relative to the posterior face of propodeum**. P: (0) leaned; (1) straight; (2) with a concavity. In the exemplars here analysed, state 0 can vary from a slight slope as in *Camponotus* species to a pronounced slope found in *Odontomachus* (Appendix 2P). State 1 is present in all *Dolichoderus* exemplars although it may be dificult to discern from state 0 before observing the object several times. An anteriorly concave petiole (state 2) is shown here in *Pachycondyla inversa* (Appendix 2S). Among ants, concavity in the anterior face of petiole is a feature only shared with *Pachycondyla curvinodis* which is probably a sister species of *P. inversa* (Mackay and Mackay 2010).

92. *Posterior face of petiole**. P/D: (0) smooth and shining with no hairs; (1) with rugosities or striae and usually hairy. In state 0 some micro villosities may be present, however a shining smooth surface is dominant. This state is discernible in *Pachycondyla crenata* and *P. carinulata*. State 1 may vary from gross striae as in *Paraponera*, to a grooved surface as in *Platythyrea*, to a finely striated face as in *Camponotus*. Variations of state 1 are coded separately in character 93.

93. *Sculpture on posterior face of petiole in the form of**. P/D: (0) fine striae horizontally arranged and simulating a fingerprint; (1) fine striae arranged randomly not forming a fingerprint-like pattern; (2) tiny grooves; (3) coarse and protruding striae. Figerprint-shaped striae are present in all *Cephalotes* and *Dolichoderus* species analysed in this study, they are usually not easy to discern due to its tiny pattern that can be confused with an apparent smooth surface when focusing light directly against the object. Randomly located striations (state 1) are found in *Cephalotes* exemplars distributed only in the outer borders of the petiole's posterior face, because most of its area is occupied by the cavity for insertion of the postpetiole. Grooves (state 2) are found in *Pachycondyla inversa*, and in *Pachycondyla* sp. 1 appressed hairs are inserted in these structures. Finally, coarse striae (state 3) are present in *Paraponera* and *Ectatomma* exemplars, these striae have a distinct arrangement in the surface, which are coded in character 94.

94. *Arrangement of striae in posterior face of petiole**. D/p: (0) no striae present; (1) horizontal; (2) concentric. A horizontal arrangement of striae is characteristic of *Ectatomma* exemplars in this study. On the other hand, a concentric arrangement was

found in *Paraponera* exemplars which possibly is a unique character state only present in this genus.

95. *Amount of horizontal striae and its arrangement in posterior face of petiole**. D/p: (0) no striae present; (1) few striae present so that a shining striae-free area is noticeable in its core, in addition these striae are relatively short so that their inner terminals (strial tips) do not intercalate with each other; (2) more striae are present than in state 0 thus, an internal gap is not discernible, these striae are long enough so that their inner tips intercalate with each other. State 1 is present in *Ectatomma tuberculatum* from the Chocó region while state 1 is present in the Amazonian *E. tuberculatum*.

96. *Orientation of striae on the inner dorsal region of third abdominal segment**. D: (0) no striae present; (1) running vertically; (2) running horizontally. Vertically orientated striae are present in *Ectatomma tuberculatum* species found the Chocó, while horizontally oriented striae are present in *E. tuberculatum* from Amazonia.

97. *Body coloration pattern**. EB: (0) a single coloration present along the body including legs; (1) concolored, either legs and body or head-abdomen and mesosoma, or mesosoma-abdomen and head are differently colored. Color variations in each segment of the body like a yellowish mallar area present in some species of *Camponotus* in this study, or lamellae in *Cephalotes,* which are colored differently from the mesosoma, are not considered in this character.

98. *Body surface**. EB: (0) opaque; (1) shining. Opaqueness and brilliancy in ants could be difficult to distinguish as it varies frequently relative to the angle of light refraction especially under artificial light. Thus it is recommended to observe the exemplar under several light angles. Hairs and striations also interfere with the degree of differentiation of this character. There are two special cases in this study, the first one is found in *Cephalotes* whose species are fully covered dorsally with tiny scale-like hairs which are brilliant, therefore strongly reflecting light, however their body surface is opaque. The other case are the exemplars in *Platythyrea* which are covered with minuscule recumbent hairs, thus forming a robe-like cover making the illusion of opaqueness.

3.2 Results Cladistic analyses

Out of the 98 characters being analyzed, 15 were recognized as parsimony uninformative. Some of these are putative autapomorphies, for example, the horizontal striations located at the posterior face of the second abdominal segment in *Ectatomma tuberculatum* (chars. 95 and 96), or the form of antennal scrobes in *Paraponera clavata* (char. 1) which so far are thought to be exclusive to this species. These "uninformative" characters were kept throughout the analyses so that they reflect an interpretation of ant morphology through phylogeny. Several of these characters could be considered as sources for synapomorphies in future studies.

The analysis under equal weights yielded 14 most parsimonious trees of 249 steps, with an average group support of 51% obtained through the Jacknife resampling. Bootstrap resamplings using 100 and 1000 replicates were also performed but the support values obtained were always inferior compared to Jacknife results, though branch arrangements in the phylogeny obtained with the strict consensus algorithm did not vary at all using both resampling methods. The amount of homoplasy calculated for the strict consensus tree under equal weights was relatively low (CI = 0.60), while the percentage of synapomorphies explaining relationships among taxa was relatively high (RI= 0.89) (Fig. 1B). The strict consensus tree was better resolved and supported than the majority rule consensus tree (not shown) which produced polytomies at the *Camponotus* and *Pachycondyla* nodes.

The eight ant genera considered in this part of the study were recovered as monophyletic under equal weights, though only 12 of the 21 terminal clades were well supported by Jacknife and Bremer values. Terminal clades mean here the related group of ant species from a given genus belonging from both geographical regions. The terminal clade formed by *Odontomachus hastatus* received a 100% support from both indexes, in contrast the *Pachycondyla crenata* clade did not get good support. As expected, most of the terminals comprising the basal genus *Camponotus* were not well supported either, one of the few exceptions is *C. sericeiventris* with 100% support in both values. Excluding the well sustented *Paraponera clavata* and *Ectatomma tuberculatum* clades which contain both a single species, the terminals in the genus *Dolichoderus* are the most firmly supported among all the group of species in this study. Internal nodes in the tree were scored with slightly lower values though most of them being above 50%.

Table 2. Parsimony sampling results showing the effect of different concavity values *K* (implied weighting scheme) for the data set of this study. Consistency (CI) and retention (RI) indexes are shown.

K	Tree Length	Trees Retained	CI	RI	Approx. No. Rearrangements (millions)
1	28.21	1	0.595	0.892	225
2	21.01	1	0.595	0.892	234
3	19.92	1	0.595	0.892	274
4	14.24	1	0.595	0.892	280
5	12.33	1	0.595	0.892	297
6	10.89	1	0.595	0.892	308
7	9.76	1	0.595	0.892	312
8	8.50	2	0.595	0.892	281
9	8.09	2	0.602	0.895	306
10	7.46	2	0.602	0.899	309

Analyses under implied weights, using concavity *K* values from 1 to 10 were very concise, yielding only one or two most parsimonious trees in every run. Generally speaking, the probability of getting most parsimonious trees is proportional to the increase of *K* (Table 2), this is, being less strict when downweighting homoplasious characters by using higher *K* values. This approach considers all characters nearly equally influential when recovering phylogenetic relationships (Goloboff 1995, Goloboff *et al.* 2008) under parsimony.

Given the results presented in Table 2 and following prior recommendations by Farris (1969), Carpenter (1988), Goloboff (1993, 1995), Carpenter (1994), Goloboff *et al*. (2008) and Keller (2011), a final sampling was performed under a high concavity value (*K*=10). Two post parsimonious trees of 7.4 steps were obtained. The strict consensus tree shown in Figure 1A has a branch support average of 53%, getting almost the same value (as compare to the tree using equal weights) for the amount of homoplasy (CI=0.602), but slightly higher amount of synapomorphies (RI= 0.9). Compared to the topology produced under equal weights, in this analysis most of the species conforming the clades produced a very similar arrangement with higher support values in average.

The differences to the tree topology under equal weights are represented by two members of the *Camponotus* clade which are encircled in red in Fig. 1A, 1B. *C. sericeiventris* is placed with confidence as sister clade to all other *Camponotus*. Under equal weights this species clade is sister to *Camponotus sp. 8* + *Camponotus sp. 2* + *Camponotus sp. 3* + *Camponotus sp. 5*, however with low support. The second and last point of incongruence between both analyses is the relationship between the clades formed by *Camponotus sp. 7* and *Camponotus sp. 6*. Under equal weights both species from each clade are clearly clustered together, while under implied weights the species in *Camponotus sp.* are separated. In any case, species arrangements under implied weights are better supported than those under equal weights.

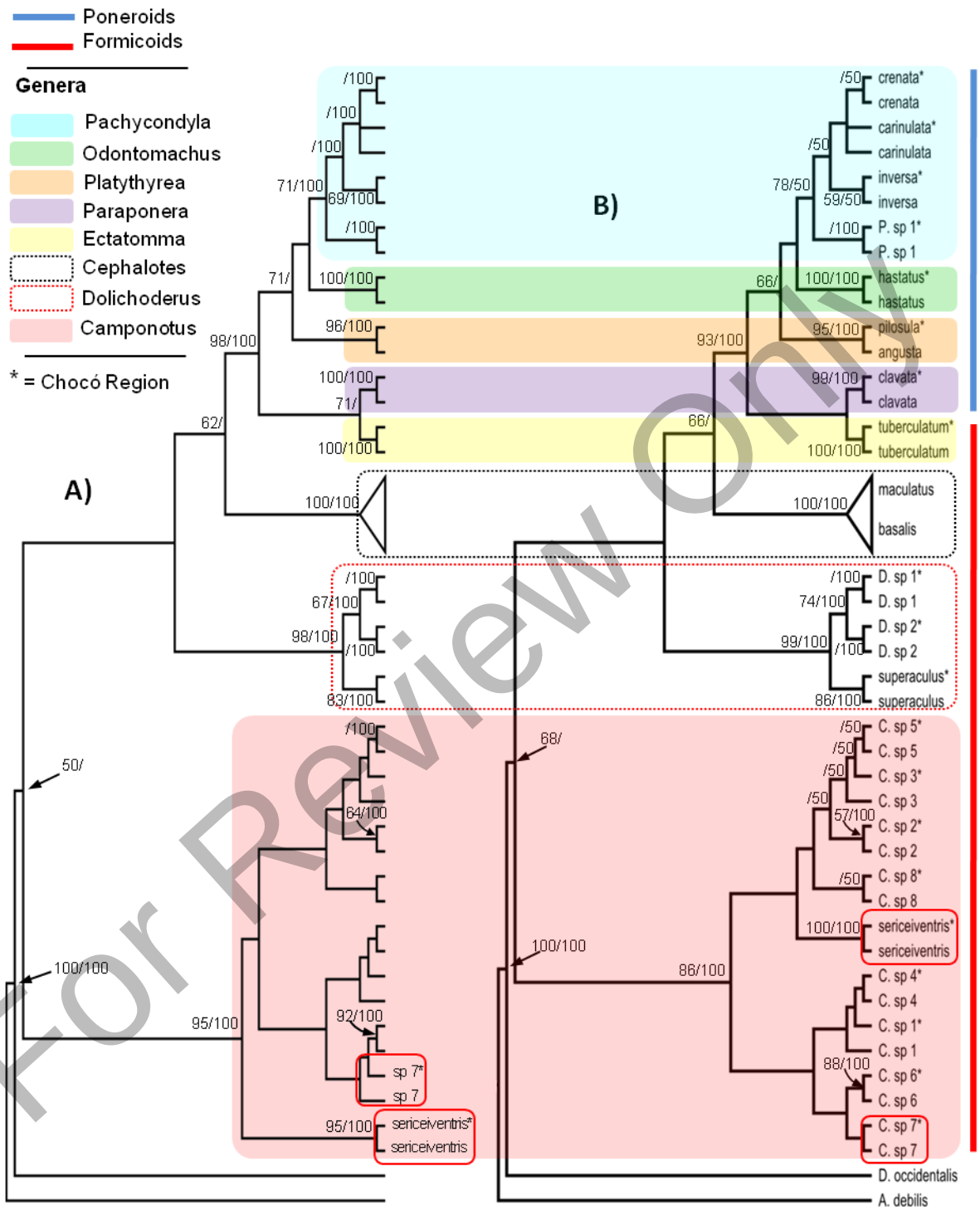


Fig. 1 Strict consensus phylogenies obtained by parsimony analyses using the complete morphological data set of 23 ant species. **A)** implied weights (concavity value *K*= 10; CI= 0.6; RI=0.9). **B)** equal weights (CI= 0.6; RI= 0.8). Species names in Figure A are omitted as clade arrangements in both trees are virtually identical, except for the species in *Camponotus* shown in red squares. The *Cephalotes* clade represents the species from both Chocó and Amazonia. Jacknife and Bremer values are provided near braches.

As noted in Fig. 1, most of the species belonging from Chocó (marked with an asterisk) and Amazonia which are represented in the tree terminals are clearly separated lineages, and the same pattern was obtained for each genus. Although not all clades were scored with high Jacknife and Bremer values, the boundaries between them are well resolved. However, two exceptions were found, first in the *Cephalotes* node where its species *C. maculatus* and *C. basalis* formed a polytomy, and second in *Pachycondyla carinulata* clade where its species relationship were not clear.

3.3 Results Molecular Analyses

As stated in the methodology just 10 species were chosen for this step of the study, those from Chocó and Amazonia, for which at least one DNA sequence was available, so as to perform the phylogenetic comparisons. The final block of sequences contained 677 bp, showing few ambiguously aligned gaps that were removed afterwards. No *indels* were detected in the formicid sequences, nor in *Atomacera debilis* (outgroup), but a three-nucleotide gap was present in *Dasymutilla occidentalis* (the second outgroup) sequence that was obtained from Genebank. Nuclear mitochondrial DNA pseudogenes (*numts*) were detected only in the sequences of the species *Pachycondyla inversa* and *Camponotus sp. 13* during the gel electrophoresis, of course these species were removed from the analyses. The amount of nucleotide base composition was AT biased, and the proportions of DNA bases through the whole block of sequences were: A 30.1%; T 38.5%; C 18.7%; G 12.7%.

Phylogenetic arrangement of most of the terminals represented in the *cox1* topology shows that species from Chocó and Amazonia are separate taxonomic entities (Fig. 2). Similar results are also depicted in the morphological phylogenies using the complete set of 23 species with equal and implied weights (Fig. 1), and the phylogeny based on the reduced data set of 10 species and 87 characters (Fig. 3).

The partitioned dataset for which the molecular phylogeny is made of (see details in methodology) contains information of all three codon positions. Although the third codon is generally under less selective pressure, acquiring more mutations through time than do both the first and second positions, which could possibly blur the phylogenetic signal as not all species in a given data set are subject to the same pace of evolution (Bofkin and Goldman 2007), here this partition summarizes quite well the phylogenetic relationships at the infraspecific level, as the majority of terminals received high support from the analyses made under Bayesian posterior probabilities, Maximum Likelihood and Maximum Parsimony.

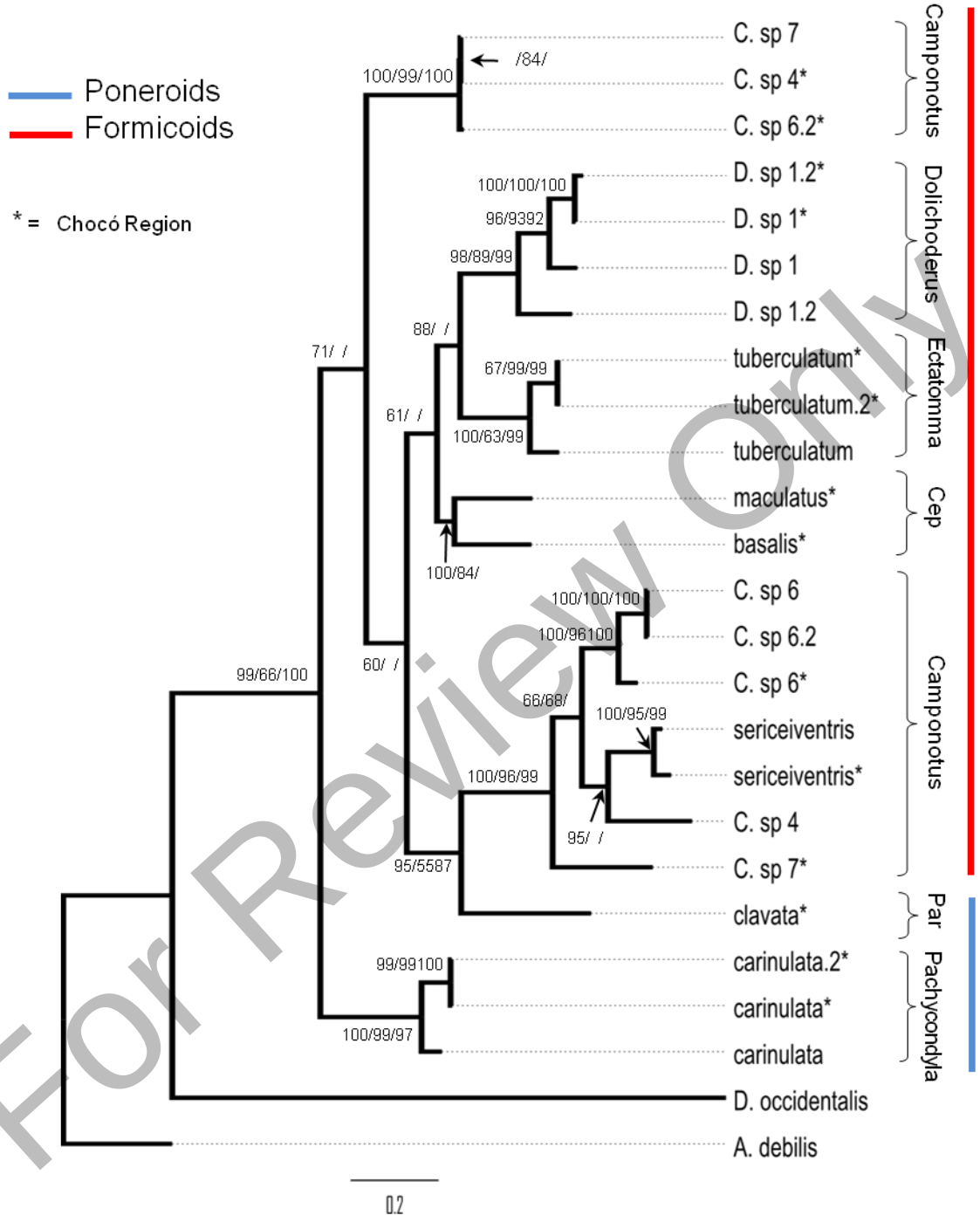


Fig. 2. Molecular phylogeny of the ant clades based on mitochondrial *cox1* sequences. Tree topology obtained from Bayesian inference and Maximum Likelihood (ML). Maximum parsimony (MP) analyses are also included. Supports near branches are posterior probabilities/ML bootstrap proportions/MP Jacknife values. Values below 50% are not shown. Ant genera are indicated to the right side of the tree. Cep, *Cephalotes*; Par, *Paraponera*.

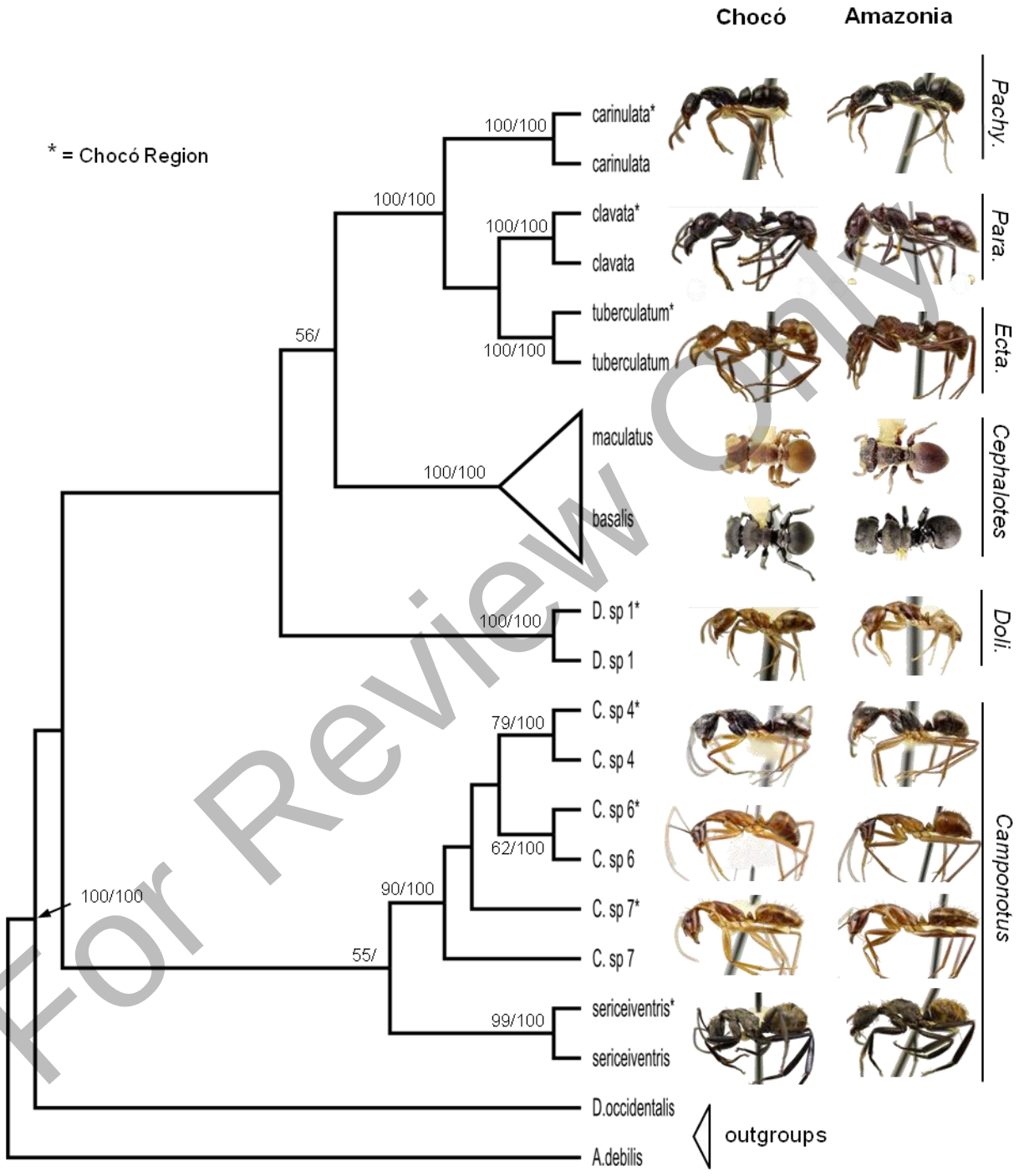


Fig. 3. Strict consensus phylogeny obtained by parsimony analysis with implied weights (*K*=10), using the morphological reduced data set of 10 species. CI= 0.70; RI= 0.85. Jacknife and Bremer values are indicated near branches. Genera are shown next to the images. Pachy, *Pachycondyla*; Para, *Paraponera*; Ecta, *Ectatomma*; Doli, *Dolichoderus*.

The tips of the tree in both morphological and molecular phylogenies are well supported and relatively well resolved without showing polytomies. Also, both approaches are congruent in defining phylogenetic boundaries between most of the species belonging from the two tropical regions located at both sides of the Andes. The *cox1* phylogeny not only illustrates these species boundaries, but also its outcome suggests that some species are not even related, an example of this is probably best depicted by *Camponotus sp. 7* and *C. sp. 4* (Appendix 2 H; 2E). *Camponotus sp. 7* belonging from Chocó is placed as the sister taxon of the clade formed by *Camponotus sp. 6* + *Camponotus sp. 4* from Amazonia + *C. sericeiventris*. Whilst the other two species, *Camponotus sp. 7* from Amazonia and *C. sp. 4* from Chocó are grouped together though only supported by ML bootstrap values. An even more interesting result is that these two species plus another exemplar from *Camponotus sp. 6* from Chocó form together a separate cluster from the rest of the species in this genus, thus suggesting a paraphyletic arrangement. In contrast, for example the clade formed by species in *Dolichoderus* is consistently supported by the molecules and morphology, it is clear therefore, that the species from Chocó are separated from those of Amazonia (Figs. 1A,1B, Fig. 2, Fig. 3).

Sites of incongruence between the morphological and molecular phylogenies are located in the internal nodes. For example, *Paraponera clavata* (Appendix 2U) is placed as sister taxon to the bigger clade of *Camponotus* in the molecular phylogeny, whereas in the morphological analyses *P. clavata* is clustered to *Ectatomma tuberculatum*, and both form the sister clade of *Pachycondyla carinulata* in the reduced data set (Fig. 3), and likewise they are sister species to *Pachycondyla, Odontomachus* and *Platythyrea* species in the complete data set (Figs. 1A, 1B). Another case is represented by the ponerine *Pachycondyla carinulata* that is located basally in the molecular phylogeny forming the sister clade to the rest of the ants examined here, whereas in the morphological analysis it is placed as a terminal clade (Figs. 1A, 1B). The location of the genus *Cephalotes* is also inconsistent between the molecules and the morphology, though in both cases with relatively low support. Finally, as mentioned before the genus *Camponotus* is recovered as paraphyletic as suggested from the molecular analyses, but is placed (with low support) as a monophyletic group in the morphological analyses.

The position of the two outgroups related to the ant species forming the crown group does not show changes across the analyses. However, root support for the formicid clade is clearly lower in all morphological phylogenies compared to the molecules.

3.4 Divergence Times Inference

In agreement to the morphological and molecular phylogenies presented before, the chronogram obtained in this study depicts the evolutionary split between most of the species belonging to Chocó from those belonging to Amazonia. Moreover, the analyses suggest the speciation process, for some of the lineages, took place during a period of intense geologic change influenced mainly by the formation of the Andes Cordillera, in Northern South America (Fig. 4).

The results illustrate a Cretaceous origin of the Formicid lineage, this is, ca. 140 Ma (highest posterior density HPD 198 - 81 Ma), being *Paraponera clavata*, in this case, the oldest taxon, basal to the rest of ants. The age of the most recent common ancestor (MRCA) to the formicids and their sister taxa (outgroups Argidae and Mutillidae) is traced back to the middle Jurassic, about 175 Ma (HPD 285 - 97 Ma). In addition, the chronogram sketches an older age for the ants with respect to the outgroups whose origin is placed in the late Cretaceous. The MRCA to the genus *Camponotus* (Formicinae) and the clade formed by *Pachycondyla* (Ponerinae) + *Cephalotes* (Myrmicinae) + *Ectatomma* (Ectatomminae) + *Dolichoderus* (Dolichoderineae) is inferred in ca. 115 Ma, of these groups, the oldest extant genera are *Pachycondyla* and *Dolichoderus* dated back to the Eocene about 55 Ma (HPD 55 - 50 Ma), the rest of extant terminal lineages have its origin in the Oligocene ca. 34 Ma.

The evolutionary split between ant lineages from Chocó and Amazonia that occurred during a period of plate tectonic readjustments spanning from ca. 30 Ma to ca. 2.5 Ma is best represented in the clades formed by *Camponotus*, *Dolichoderus* and *Ectatomma*. In the case of the latter genus, for example *E. tuberculatum* from Chocó, diverged from its sister Amazonian species approximately 5 Ma, which coincides with a period of intensified North Andean mountain building during the late-middle Miocene (ca. 12 Ma) to early Pliocene (~4 Ma) (Mora *et al*. 2010). Furthermore, these geologic events overlapped with the formation of a large aquatic environment known as the Pebas system that covered a broad proportion of Northern “pan-Amazonia” (Hoorn *et al*. 2010, Riff *et al*. 2010). Therefore, these series of landscape disturbances promoted allopatric speciation of the fauna (and possibly flora) of the ancient “pan-Amazonia”, aspect that is reflected in the chronogram obtained here.

In addition, the dated phylogram shows some differences in the internal phylogenetic arrangement of nodes compared to the previous phylogenies obtained in this study. The placement of *Paraponera clavata* as the oldest ant species within the ingroup is the main difference, which in contrast to the morphological results, this species is placed either close to the tips of the tree, or in a middle node in the phylogenies based on the reduced (Fig. 3) and complete (Figs. 1A, 1B) data sets, respectively. In the phylogeny shown in Fig. 2 *P. clavata* is placed as the sister taxon of the paraphyletic genus *Camponotus*, an evolutionary relationship not obtained before among studies of ants evolution. Another point of discrepancy is the basal placement of the subfamily Formicinae (e.g. the genus *Camponotus*) in the morphological analyses, whereas in both molecular phylogenies (Figs. 2, 4) this genus is placed amongst the youngest clades.

In the chronogram, the ponerine *Pachycondyla carinulata* appears more closely related to *P.clavata* than in the molecular phylogeny in Fig. 2. The relationship between these two lineages has been supported before in other studies (e.g. Astruc *et al.* 2003, Moreau *et al.* 2006, Rabeling *et al.* 2008) and is widely accepted that these species and their allies form basal old groups among the Formicidae, aspect that is also reflected in Fig. 4. On the other hand, the morphological analyses in thi study show a similar relationship between these two species, though both are placed close to the tree terminals.

The dated phylogeny based on “relaxed clock” assumptions was compared to that obtained with “strict clock” assumptions. The tree topology resulting from the latter (not shown) is very similar to that obtained using relaxed clock assumptions. The only two differences are, on one hand, the placement of *P. clavata* as the sister lineage to *Camponotus* as inferred by the assumption of a constant rate of evolution (strict molecular clock). On the other hand, this latter approach places the origin of the Formicidae at the beginning of the early Cretaceous, which is about 40 million years younger than by using a relaxed molecular clock (Fig. 4).

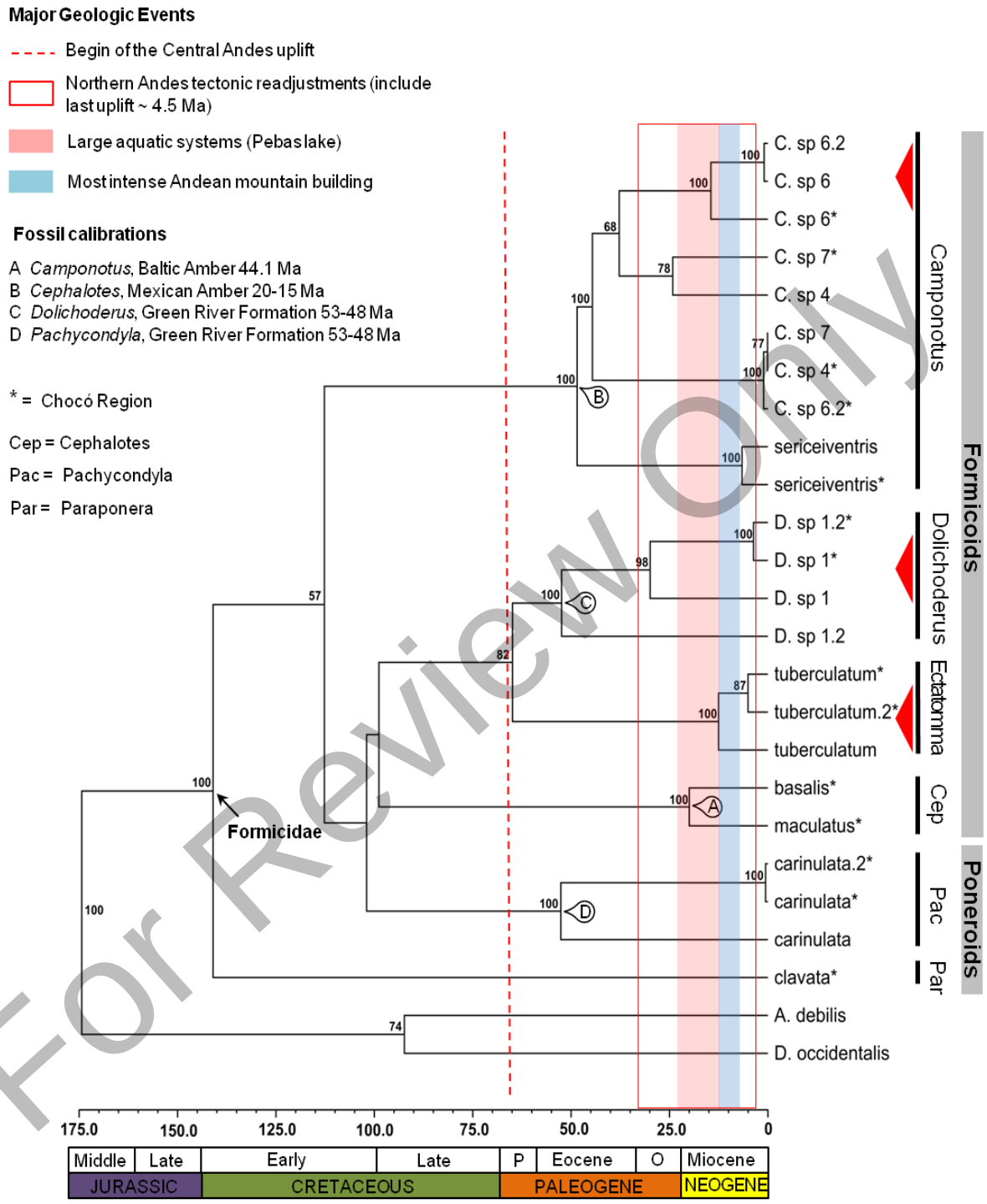


Fig. 4. Phylogram based on the reduced data set of 10 ant species assuming uncorrelated nucleotide substitution rates among lineages. Posterior probabilities above 50% are indicated on branches. Red marks next to species names show the most probable cases for which allopatric speciation between species from Chocó and Amazonia was influenced by major geologic events. Cep. *Cephalotes*; Pac, *Pachycondyla*; Par, *Paraponera*.

New Species

The results shown here demonstrate that most of the target species from Chocó and Amazonia have undergone phyletic divergence through time possibly influenced, among other factors, by the formation of the Andes chain. Most of the individuals identified to species level in this study are confirmed by the following sources: revision of the original descriptions of most type species; physical comparisons to other identified specimens in the MEPN and ZSM Collections; digital data bases in internet curated by ant spetialists; detailed morphological re-examination of several individuals per species (Table 1); information on geographic distribution published by spetialists in monographic revisions (see references in methodology), as well as professional internet sources (see details in methodology). Exceptions where still some taxonomic uncertainty remains are: *Cephalotes basalis* from Amazonia, *Cephalotes maculatus* from Chocó, and *Pachycondyla crenata* from both regions.

Based on the whole set of arguments presented in this work, i) taxonomical information; ii) phylogenetics; and iii) divergence times explaining the lineages split with reference to ancestral major changes in landscape, I propose that the following five species (out of the 10 taxonomically confirmed) are new to science: a) *Camponotus sericeiventris* from Chocó; b) *Dolichoderus superaculus* from Amazonia, c) *Ectatomma tuberculatum* from Chocó; d) *Odontomachus hastatus* from Chocó; e) *Pachycondyla carinulata* from Chocó.

These new species are to be described in a future study following further molecular and morphological analyses of more specimens and final veredict of the specialists in each genus.

4. Discussion and Conclusions

Internal Phylogenetic Relationships

Though the aim here was not to provide a further understanding of the current internal classification of the Formicidae, nor clarify the still debatable phylogenetic position of some of the currently recognized subfamilies and genera, the phylogenetic relationships at the supraspecific level (subfamilies) obtained in this study through morphological analyses majorly resemble important synthetic studies of Brown (1954), Wilson *et al.* (1967), Taylor (1978), Baroni Urbani (1989), Baroni Urbani et al. (1992), and Keller (2011), made so far on the evolutionary classification of ants, all of them based on morphology as well. Notwithstanding the relatively small number of species used here (in comparison to Keller 2011, for example) representing a small fraction of the 21 ant subfamilies (Bolton 2003, Bolton *et al.* 2006), the results shown here have achieved in reconstructing a well resolved phylogeny representing a part of the whole formicid clade.

Nonetheless, not few disagreements between those previous studies arose during a period of intense rearrangement of the internal classification of ants. The scant inconsistencies of the phylogenetic classification shown here with respect to the most-up to-date studies, without a doubt, can be explained mostly by the difference in sample size (number of species representing the subfamilies and/or supraspecific taxa). As stated by Prendini (2001), variations in sample size from study to study may lead proportionally to different conclusions. Significantly increasing the number of taxa, say by including all or most of constituent species of a given group, can have a profound [probably negative] effect on the accuracy of the analyses (Hendy and Penny 1989, Wheeler 1992, Kim 1996, Poe and Swofford 1999). Though is desirable to include as many species available for analyses, it may be impractical [sometimes even intractable with current technology] if that number represents close to the total number of species described for a given group (Donoghue 1994, Nixon and Carpenter 1996, Rice *et al*. 1997), especially if it is a megadiverse one. On the other side, including too few representatives of the group of interest may lead also to differing conclusions (Prendini 2001). In any case, according to Wiens (1998: 411), while comparing the performance of ground plans vs. exemplars in parsimony analyses, he concluded that: “using species as terminals gives consistently more accurate phylogenetic estimates than do other coding methods, even when only few

species are sampled from each higher taxon". At some extent, the results shown here reflect Wiens (1998) conclusions.

In the present study, the methodologies, e.g. number of replicates during data resampling, or type of parsimony algorithm employed, have less influence than sample size on the resulting most parsimonious topologies, as all rely on the same principle, this is, to reach a minimum possible number of evolutionary steps given the characters provided. In this study, more than 60% of the characters were taken mainly from the references indicated before. The rest of coded characters are novel, but most of them represent autapomorphies so that, they reflect relationships between the species clades of concern. In this sense, it was expected that the morphological phylogeny provided here agrees with current phylogenetic thought about subfamily relationships in the Formicidae.

A relatively different internal arrangement was produced by the molecular analyses presented in Fig. 2 as the subfamily relationships are "disturbed" by two main aspects. First, the close relationship of a basal lineage to a higher clade, and second the presence of a paraphyletic group. The former case refers to the placement of *Paraponera clavata* (Paraponerinae) close to several species of *Camponotus* (Formicinae). *P. clavata* is considered today one of the basal lineages to all ants (Bolton *et al.* 2006, Moreau *et al.* 2006, Ward 2007) thus, may not be congruent to group this species as sister to *Camponotus* (Formicinae) whose phylogenetic placement is broadly considered to be among the youngest ant clades (Bolton *et al.* 2006, Moreau *et al.* 2006, Ward 2007). That unusual grouping is also not supported by the morphological analyses obtained here, where *P. clavata* is assigned as sister to *Ectatomma tuberculatum* (Ectatomminae), however, this latter phyletic association is also not in agreement to most of up-to-date molecular phylogenies like that proposed by Moreau *et al.* (2006) who employed a large number of species and genes.

Secondly, the placement of *Camponotus* as paraphyletic (with relatively high support) may confound within-tree relationships at the subfamily and genera level, as for example in this case, one of the branches of this genus is closer to "poneromorph" taxa (*Paraponera clavata* and *Pachycondyla carinulata*) than to other formicoids (like e.g. *Dolichoderus*) which are supposed to be more related to the subfamily Formicinae to which *Camponotus* belongs. In spite of this, there are some evidences yielded by DNA analyses (Brady *et al.* 2000) and morphology (Mackay 2007) suggesting that this genus would be in deed

paraphyletic. The paramount species richness of *Camponotus* (~ 1.000 described worldwide, Bolton 2006) together with an accentuated worker polymorphism (Fernández 2003) present in many species which have troubled tentative classifications like that of Mackay (2007), have temporarily blocked, thorough phylogenetic analyses of this group.

Without considering the unusual phylogenetic placement of *P. clavata* and the paraphyly of *Camponotus*, the rest of the internal phylogenetic arrangements shown in the molecular topology are in agreement to current major molecular phylogenetic studies like that of Moreau *et al.* (2006) and Rabeling *et al.* (2008). It is likely that the present few inconsistencies are due to the use of just one gene and also because of the difference in sample size related to the inclusion of species representing other ant genera, however, as expressed before, the aim of this study is to clarify evolutionary relationships at the species level, regardless of the placement of deeper nodes in the tree, for example at the level of subfamilies. Moreover, the phylogenetic information provided by *cox1* is not usually reliable to explain deep divergencies (Galtier *et al.* 2009, Pacheco *et al.* 2011) but, as demonstrated in this study, is arguably accurate in defining species boundaries. In addition, is quite likely that the phylogenetic arrangement at the supraspecific level changes (and possibly becomes more clear) through the inclusion of many more species and also more genera representing other ant subfamilies, it has happened before in ant phylogenetic studies (see in sequence Astruc *et al.*, 2003, Moreau *et al.* 2006, Ward 2007, Rabeling 2008), as well as in several other groups of organisms like birds for example (see Cooper and Penny 1997, van Tuinen and Hedges 2001, Brown *et al.* 2007, Pacheco *et al.* 2011)

Different Species in Chocó and Amazonia

At the specific level, both morphological and molecular phylogenies produced here are quite consistent between each other, and in most cases well supported by resampling values. I refer here to *consistency*, to the same stable phylogenetic arrangement of the terminals being continuously obtained as the sampling across the tree space increases. These results are the first starting argument in favor of the hypothesis of speciation between the taxa considered from both geographic regions, and it means ultimately, that most of the previously considered single biological entities (species), are in fact, in some cases sister lineages (e.g. *Ectatomma tuberculatum*), and in other cases they are possibly not even related (e.g. *Camponotus* sp. 7, see Fig. 2) despite being virtually identical in

morphology (Appendix 2H and Appendix 4). These arguments are supported by direct observations of the specimens and independently of the parsimony morphological results, indicate evidences of dissimilarities in most of the target species (see Fig. 3 and Appendix 4). Additionally, by examining the branch lengths and genetic distances of these closely related taxa, I suggest here that speciation may have occurred relatively recent, aspect that is supported by molecular clock analyses which is discussed below.

Given the congruence between the molecules and morphology at the specific level it is evident that a general pattern of lineage divergence is present among the majorority of ants studied here. If this pattern can be confirmed for even more ant species in northern South America, then it may be very likely to found it in other insect groups like the Coleoptera or Diptera that are also quite abundant especially in the tropical canopy (Erwin 1983, 1988)

Unfortunately there was not enough amount of evidence for every single species, as in some cases, DNA sequences were needed for some species like e.g. *Pachycondyla* and *Odontomachus* in both regions, and in other cases related to the morphological analyses the tree topology did not show clear lineage divergencies supported by strong resampling values, for example in some species of *Camponotus*. However, in some of these situations both approaches counterbalanced each other, for example species in *Camponotus* sp. 7 and *Camponotus* sp. 4 rare not well supported by parsimony values in the morphological analysis using the complete data set of 23 species (Fig. 1), but in constrast these species clades were higly scored under the molecular analyses (Figs. 2 and 4), but also when examining the reduced morphological data set (Fig. 3). Higher parsimony values in the latter with respect to the analysis using the complete data set are likely due to the removal of some non informative characters indicated in Appendix 3 by asterisks.

Another point of support for the present arguments may be gathered through the ecology of each species, by making repeated and continuous observations of different ant colonies in both regions. Obviously, this is an enormous task, which extends far beyond the objectives of this work. Currently, there are very few studies on ant evolution where ecological traits have been included in the phylogenetic analyses, for example, Baroni Urbani (1989) who was the first that introduced ecological data together with the morphology in a cladistic study, considered in his analyses the use of silk in ants for the construction of the nest, social parasitism, nomadic habits, among other general

characteristics which are present in not just one or two species but in many ant species in several genera across the Formicidae (Hölldobler and Wilson 1990). Clearly, by including those characters while analyzing species relationships, no support can be provided to the terminals in the resulting phylogeny. Similarly to other megadiverse insect groups, the ecology of most ant species known to date is still far from being understood after more than a centrury of research (Wilson 2010). On the one hand, the inclusion of general ant behaviors like those used by Baroni Urbani (1989) in ant phylogenetics does not provide enough information to examine species boundaries. On the other hand, in order to study the ecology of the present species would require a long process, driven mainly by the uncertainty in getting economic support for carrying out the research.

For those reasons, plus the facts that access to both geographical regions is quite difficult, and that most of the species considered here have arboreal habits (*pers. obs.,* but see also Hölldobler and Wilson 1990, Bolton 2003, Fernández 2003, Mackay and Mackay 2010) which make ecological observations even harder to pursue in a short term, the results shown here, though still preliminary, as there is additional molecular information to be added (see methodology), can be considered the first important set of clues supporting the hypothesis of lineage divergence between ant species from Chocó and Amazonia in northern South America.

Lineages Divergence Influenced by Geologic Events

Molecular analyses illustrated in the chronogram (Fig. 4) suggests that at least six out of 10 formicid species clades may have undergone phyletic split across the Oligocene, Miocene and Pliocene during a period of approximately 30 million years. Some species belonging from Chocó like *Pachycondyla carinulata* and *Dolichoderus* sp. 1 may even have diverged from their sister Amazonian species during the Pleistocene-Holocene epochs in the Quaternary (~2.5 Ma). According to a recent study by Hoorn *et al.* (2010) the Andes chain uplift in northern South America began some 65 to 33 Ma. During this period, the so called “pan Amazonian” cratonic-dominated landscapes changed to Andean-dominated landscapes. Before the Central (Perú and Bolivia) and Northern (Ecuador, Colombia and Venezuela) Cordilleras reach their current elevation ranges ca. 2 Ma, tropical pan Amazonian forests covered a much wider geographical range than today’s Amazonian area (Albert *et al.* 2006, Hoorn *et al.* 2010). In the process of Andean uplift, several geographic regions became isolated. In northern South America, two of

those regions were the Pacific Chocó block and the western Amazonia, both regions under intense geologic activity that began in the early-middle Oligocene around 30 Ma (Duque-Caro 1990, Kellog and Vega 1995, Gregory-Wodzicki 2000, Hoorn *et al.* 2010). This probably was the first major event initiating the process of lineage split between organismic populations (aquatic and terrestrial) as large terrain areas became gradually isolated, but also in parallel, it created new montane areas that coincide with the diversification of modern plant and animal Andean and sub-Andean genera (Hoorn *et al.* 2010).

During the Oligocene the Pebas aquatic system (see Nuttal 1990) whose formation started with a continental break in northern South America probably driven by tectonic events and foreland-intracratonic basin subsidence (Wesselingh *et al.* 2006), was an extensive > 1 million Km^2 fresh water system of long-live lakes that occupied a major part of western pan Amazonia from ca. 23 to ca. 8 Ma (Nuttal 1990, Wesselingh *et al.* 2006, Hoorn et al 2010). This aquatic system provided the elements for endemic radiations of molluscs and ostracods but at the same time restricted migration of the biota distributed in the east part of pan Amazonia from that distributed across the Chocó block, west-central pan Amazonia (today's Perú), and at the emerging western Andes (Cooper *et al.* 1995, Villamil 1999, Wesselingh *et al.* 2006, Hoorn et al 2010). According to the information provided by the mentioned authors prior to the formation of the Pebas system a wide extension of tropical pan Amazonian forests exited, thus allowing the evolution of terrestrial organisms. Since ants were present in pan Amazonian forests much before both the Andes emergence and the formation of the Pebas system (present results), then it is quite possible that ancient formicid populations were isolated at varying degrees of intensity by those events, which ultimately led to lineage divergence. The clearest cases supporting this hypothesis are depicted in the clades of *Caponotus*, *Dolichoderus* and *Ectatomma*, where species from Chocó diverged from their Amazonian sister lineages around the end of the Miocene coinciding with the mentioned geological periods that dramatically modifyied large areas of north-central South America, and almost certainly the evolution of its ancient biota.

About the New Species and Considerations for the Evolution of Cryptic Taxa

The number of new species proposed in the results is relatively low related to the complete set analyzed here. One must consider that the taxa in *Camponotus* were not confirmed to the species level however, it is very likely that at least half of them (not counting *C.*

sericeiventris which is already proposed as new taxon) are also new to science. Likewise most of the ant species in this study, I found genetic and morphological differences between species from both regions. In the same way, regarding the confirmed species that are not proposed as new taxa, I must refer here to *Paraponera clavata*, *Pachycondyla inversa* and *P. crenata* for which unfortunately not enough evidences were accumulated in this study to propose a new taxonomic status.

The case of the cryptic *P. inversa* for example, where apparently no morphological differences were found between examplars from both regions, and for which final molecular results are still pending, illustrates similar cases of crypticism in this study and across the entire family as well, which may be very common according to Seifert (2009). This case also suggests that [at least] two scenearios may explain a departure from the hypothesis of speciation driven by large changes in landscape. First, gene flow between populations of these species from Chocó and Amazonia has not stoped despite the influence of geographical barriers. This may not be surprising as this is the case of many insect species with broad distributional ranges (Spitzer and Leps 1989, Murphy and Breed 2007, Alpizar *et al.* 2012) capable to adapt to a wide variety of different habitats and climates as they search for new places to live (Tauber *et al.* 1986). *Pachycondyla inversa* itself is widely distributed across Central and South America, from southern Mexico to southern Brazil (Mackay and Mackay 2010). Is not known however, if this species in deed have the ability to overpass large natural barriers and/or adapt to different habitats through its migration path searching for nesting places. Moreover, molecular phylogenies and population genetics methods, based on several exemplars per species collected in several localities, are still required to elucidate species boundaries in the context of phylogeography.

A second scenario involving ecological traits through the study of the natural history of a given cryptic species may introduce quite valuable additional information about the species evolution. Ants are well known to interact in several ways with a huge variety of organisms (Hölldobler and Wilson 1990. Bronstein 1998, Christian 2001, Delabie *et al.* 2003, Delabie and Fernández 2003), including themselves for example in social parasitism or slave making behaviors (Foitzik *et al.* 2001). The nature and frequency of these interactions may influence dramatically the natural history of ants and as extension, their evolution (Davidson 1998, Ness *et al.* 2010). Since it is very likely that populations of a given ant species cannot find the same amount and “types” of organisms with which they interact

ecologically, then it is also likely that these populations evolve in a different way in response to different ecological interactions. However, being involved in different forms of interactions does not necessarily imply a morphological change which obviously, depends on the frequency, type, duration across time, and intensity of the interaction. A case probably illustrating this issue is brought by the species *Pachycondyla villosa* (related to *P. inversa* in this study) which is known throughout America, from southern United States to Brazil and Argentina. It has been reported that this species nests exclusively in bromeliad epiphytes in México, defending its hosts from other insect threats (Dejean 1990), given this interaction is likely that the populations of this species evolve differently through time in contrast to other populations of the "same recongnized" species which nest for example in dead trunks in the ground (*pers. obs.,* Dejean and Corbara 1998, Mackay and Mackay 2010). As reported by Seifert (2009), *P. villosa* was the focus of intense studies in different subjects of biology from 1984 to 2002 before it was recognized to consist of three different species (Lucas *et al.* 2002), possibly all of them not showing the same ecological traits.

Current phylogenetic, taxonomic and ecological research in various families comprising the order Hymenoptera have made clear the fact that crypticism is much more usual than previously expected (Seifert 2009, Heraty 2009). If further research on ant species continue to support this fact then is possible that current monographic revisions related to the Formicidae (showing large geographic ranges for a single species like *Pachycondyla villosa*, *sensu* Mackay and Mackay 2010) need to be re-revisited but with the aid of a multi-method approach (morphology, DNA, allozymes, cuticular hydrocarbons, etc) to enhance today's state of alpha taxonomy.

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7. Appendixes

Appendix 1. List of species examined. Museum vouchers (Museo Escuela Politécnica Nacional MEPN, Quito, Ecuador) and collection localities are included.

Species	Chocó		Amazonia	
	Voucher	Locality	Voucher	Locality
Camponotus sericeiventris	1559	Pajonal	1421	Guiyero, L. Guaorani
Camponotus sp. 1	1556	Pajonal	1418	Guiyero, L. Guaorani
	1562	Salto del Bravo	1446	Guiyero, B. Nambay
Camponotus sp. 2	1557	Pajonal	1421	Guiyero, L. Guaorani
			1246	Bermejo
Camponotus sp. 3	1037	Salto del Bravo	1361	Cuyabeno
Camponotus sp. 4	1556	Pajonal	1421	Guiyero, L. Guaorani
	1565	Salto del Bravo		
Camponotus sp. 5	1556	Pajonal	1361	Cuyabeno
	1020	Salto del Bravo	1311	Guiyero, L. Tigre
Camponotus sp. 6	1037	Salto del Bravo		
	1563	Salto del Bravo	1443	Guiyero, B. Nambay
	1566	La Tabla		
Camponotus sp. 7	1568	La Tabla	1313	Guiyero, L. Tigre
Camponotus sp. 8	1570	La Tabla	1445	Guiyero, B. Nambay
Cephalotes basalis	1556	Pajonal	3711	Tiputini
	4688	Charco Vicente		
			1249	Bermejo
Cephalotes maculatus	1564	Salto del Bravo	1412	Guiyero, L. Guaorani
			1518	Jatun Sacha
Dolichoderus superaculus	1568	La Tabla	1361	Cuyabeno
Dolichoderus sp. 1	1557	Pajonal	1281	Guiyero, L. Tigre
Dolichoderus sp. 2	1559	Pajonal	1421	Guiyero, L. Guaorani
Ectatomma tuberculatum	1557	Pajonal	5319	Onkonegare
			5320	Onkonegare
			5322	Onkonegare
			5323	Onkonegare
Odontomachus hastatus	1012	Salto del Bravo	1421	Guiyero, L. Guaorani
	1037	Salto del Bravo		
Pachycondyla carinulata	1556	Pajonal	1295	Guiyero
Pachycondyla crenata	1556	Pajonal	1361	Cuyabeno
			1421	Guiyero, L. Guaorani
Pachycondyla inversa	1556	Pajonal	1295	Guiyero
	4876	Pajonal		
Pachycondyla sp.1	1037	Salto del Bravo	1446	Guiyero, B. Nambay
	1100	La Tabla		
Platythyrea angusta			1299	Guiyero
Platythyrea pilosula	4833	Salto del Bravo		
Paraponera clavata	1563	Salto del Bravo	1093	Tiputini

Appendix 2. Species images. Museum (MEPN) vouchers are shown next to the species labels.

Chocó

A1) *Camponotus sericeiventris* MEPN 1559

Amazonia

A2) *Camponotus sericeiventris* MEPN 1421

B1) *Camponotus* sp 1 MPEN 1562

B2) *Camponotus* sp 1 MPEN 1446

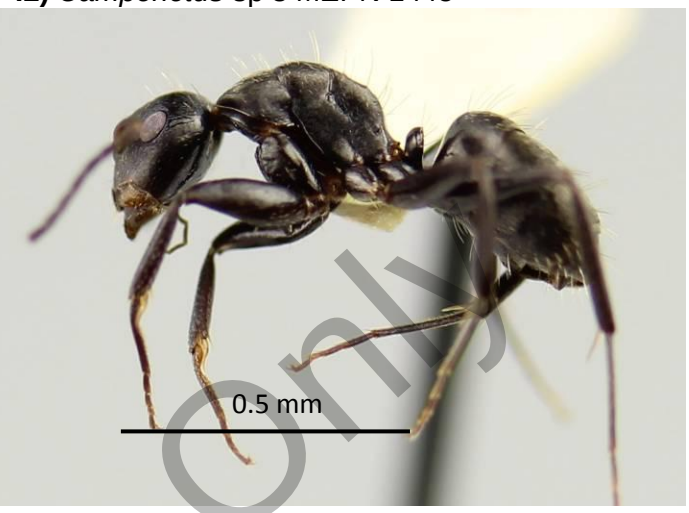
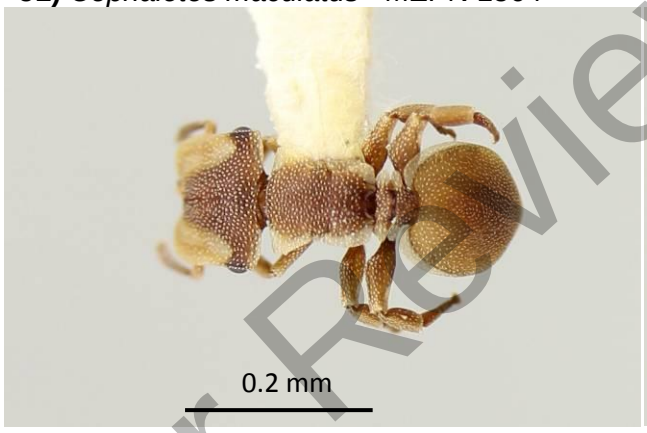
Appendix 2 (*continued*)

Chocó	Amazonia
C1) *Camponotus* sp 2 MEPN 1557	**C2)** *Camponotus* sp 2 MEPN 1421
	
D1) *Camponotus* sp 3 MEPN1037	**D2)** *Camponotus* sp 3 MEPN 1445
	
E1) *Camponotus* sp 4 MEPN 1556	**E2)** *Camponotus* sp 4 MEPN 1421
	

Appendix 2 (*continued*)

Chocó	Amazonia
F1) *Camponotus* sp 5 MEPN 1570	**F2)** *Camponotus* sp 5 MEPN 1248
	
G1) *Camponotus* sp 6 MEPN 1563	**G2)** *Camponotus* sp 6 MEPN 3111
	
H1) *Camponotus* sp 7 MEPN 1568	**H2)** *Camponotus* sp 7 MEPN 1313
	

Appendix 2 (*continued*)

Chocó	Amazonia
I1) *Camponotus* sp 8 MEPN 1570	**I2)** *Camponotus* sp 8 MEPN 1445
	
J1) *Cephalotes maculatus* MEPN 1564	**J2)** *Cephalotes maculatus* MEPN 1412
	
K1) *Cephalotes basalis* MEPN 4688	**K2)** *Cephalotes basalis* MEPN 3711
	

Appendix 2 (*continued*)

Chocó	Amazonia
L1) *Dolichoderus superaculus* MEPN 1568	**L2)** *Dolichoderus superaculus* MEPN 1249
	
M1) *Dolichoderus* sp. 1 MEPN 1557	**M2)** *Dolichoderus* sp. 1 MEPN 1281
	
N1) *Dolichoderus* sp. 2 MEPN 1559	**N2)** *Dolichoderus* sp. 2 MEPN 1421
	

Appendix 2 (*continued*)

Chocó	Amazonia
O1) *Ectatomma tuberculatum* MEPN 1557	**O2)** *Ectatomma tuberculatum* MEPN 5319
	
P1) *Odontomachus hastatus* MEPN 1012	**P2)** *Odontomachus hastatus* MEPN 1421
	
Q1) *Pachycondyla carinulata* MEPN 1556	**Q2)** *Pachycondyla carinulata* MEPN 1295
	

Appendix 2 (*continued*)

Chocó	Amazonia
R1) *Pachycondyla crenata* MEPN 1556	**R2)** *Pachycondyla crenata* MEPN 1249
	
S1) *Pachycondyla inversa* MEPN 1556	**S2)** *Pachycondyla inversa* MEPN 1295
	
T1) *Pachycondyla* sp. 1 MEPN 1037	**T2)** *Pachycondyla* sp. 1 MEPN 1446
	

Appendix 2 (*continued*)

Chocó	Amazonia
U1) *Paraponera clavata* MEPN 1563	**U2)** *Paraponera clavata* MEPN 1093
	
V1) *Platythyrea pilosula* MEPN 4833	**V2)** *Platythyrea angusta* MEPN 1299
	

Appendix 3. Morphological character matrix. Species marked with an asterisk belong to Chocó region. Additive (ordered) characters are indicated with "O", while unordered characters with "U". Characters omitted for the reduced data set (see methodology) are indicated with an asterisk. "**–** " = inapplicable; "?" = character states not observed

	1				5					10					15					20					25					30
		O		U		O	O	U		O	O			U	O	U								O				O	
*Dolichoderus sp 1**	1	1	0	0	1	1	0	1	0	1	1	0	0	0	0	2	0	1	0	1	1	0	1	0	1	0	-	0	0	-
Dolichoderus sp 1	1	1	0	0	1	1	0	1	0	1	1	0	0	0	0	2	0	1	0	1	1	0	1	1	1	0	-	0	0	-
*D. superaculus**	1	1	0	0	1	1	0	1	0	1	1	0	0	0	0	2	0	1	0	1	1	0	1	0	1	0	-	0	0	-
D. superaculus	1	1	0	0	1	1	0	1	0	1	1	0	0	0	0	2	0	1	0	1	1	0	1	0	1	0	-	0	0	-
*Dolichoderus sp 2**	1	1	0	0	1	1	0	1	0	1	1	0	0	0	0	2	0	1	0	1	1	0	1	0	1	0	-	0	0	-
Dolichoderus sp 2	1	1	0	0	1	1	0	1	0	1	1	0	0	0	0	2	0	1	0	1	1	0	1	0	1	0	-	0	0	-
*Ectatomma tuberculatum**	1	1	1	2	1	1	1	4	0	1	0	0	1	1	0	0	1	1	0	1	1	1	1	2	1	0	-	0	2	-
Ectatomma tuberculatum	1	1	1	2	1	1	1	4	0	1	0	0	1	1	0	0	1	1	0	1	1	1	1	2	1	0	-	0	2	-
*Camponotus sericeiventris**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
Camponotus sericeiventris	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
*Camponotus sp 1**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
Camponotus sp 1	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
*Camponotus sp 2**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	0
Camponotus sp 2	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	0
*Camponotus sp 3**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	0
Camponotus sp 3	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	0
*Camponotus sp 4**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
Camponotus sp 4	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
*Camponotus sp 5**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	0
Camponotus sp 5	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	0
*Camponotus sp 6**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	2	-
Camponotus sp 6	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	2	-
*Camponotus sp 7**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	2	-
Camponotus sp 7	1	2	0	0	1	0	-	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	-	0	2	-
*Camponotus sp 8**	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
Camponotus sp 8	1	2	0	0	1	0	-	1	0	0	0	0	0	0	2	0	0	1	0	1	1	1	1	0	0	0	-	0	1	1
*Cephalotes maculatus**	1	2	1	2	1	1	2	0	1	0	-	1	0	0	2	0	0	1	0	1	1	1	1	1	1	0	-	0	0	-
Cephalotes maculatus	1	2	1	2	1	1	2	0	1	0	-	1	0	0	2	0	0	1	0	1	1	1	1	1	1	0	-	0	0	-
*Cephalotes basalis**	1	2	1	2	1	1	2	0	1	0	-	1	0	0	2	0	0	1	0	1	1	1	1	1	1	0	-	0	0	-
Cephalotes basalis	1	2	1	2	1	1	2	0	1	0	-	1	0	0	2	0	0	1	0	1	1	1	1	1	1	0	-	0	0	-
*Paraponera clavata**	0	2	1	2	1	1	0	3	0	0	2	0	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	0	0	-
Paraponera clavata	0	2	1	2	1	1	0	3	0	0	2	0	1	1	0	1	0	1	0	1	1	1	1	0	1	1	0	0	0	-
*Odontomachus hastatus**	1	0	1	1	1	2	-	2	0	2	1	0	1	1	1	0	0	0	1	1	1	1	1	0	1	0	-	0	0	-
Odontomachus hastatus	1	0	1	1	1	2	-	2	0	2	1	0	1	1	1	0	0	0	1	1	1	1	1	0	1	0	-	0	0	-
*Pachycondyla carinulata**	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
Pachycondyla carinulata	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
*Pachycondyla inversa**	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
Pachycondyla inversa	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
*Pachycondyla crenata**	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
Pachycondyla crenata	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
*Pachycondyla sp 1**	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
Pachycondyla sp 1	1	0	1	1	1	2	-	2	0	3	1	0	1	1	0	0	0	1	0	1	1	0	1	2	1	0	-	1	0	-
*Platythyrea pilosula**	1	2	1	1	1	2	3	2	0	0	1	0	1	2	0	0	0	1	0	1	1	1	1	0	1	0	-	0	0	-
Platythyrea angusta	1	2	1	1	1	2	3	2	0	0	1	0	1	2	0	0	0	1	0	1	1	1	1	0	1	0	-	0	0	-
Dasymutilla occidentalis	-	-	0	0	0	-	-	-	0	0	-	-	1	0	3	0	0	1	0	0	1	1	0	1	1	0	-	0	0	-
Atomacera debilis	-	-	0	0	0	-	-	-	0	0	-	-	0	0	3	0	0	0	1	1	0	0	0	1	1	0	-	0	0	-

Appendix 3. (*continued*)

						3				4						4				5				5				6
						5				0						5				0				5				0
	U					O	O	O	O							O	U			U			O			O				O
*Dolichoderus sp 1**	2	-	1	0	0	0	0	0	1	1	0	1	1	1	0	1	1	0	0	0	0	2	1	0	3	1	1	0	0	0
Dolichoderus sp 1	2	-	1	0	0	0	0	0	1	1	0	1	1	1	0	3	1	0	0	0	0	2	1	0	3	1	1	0	0	0
*D. superaculus**	2	-	1	1	0	0	0	0	1	1	0	1	1	1	0	3	1	0	1	0	0	2	1	0	3	1	1	0	0	0
D. superaculus	1	-	1	1	0	0	0	0	1	1	0	1	1	1	0	3	1	0	1	0	0	2	1	0	3	1	1	0	0	0
*Dolichoderus sp 2**	2	-	1	0	0	0	0	0	1	1	0	1	1	1	0	3	1	0	2	0	0	2	1	0	3	1	1	0	0	0
Dolichoderus sp 2	2	-	1	0	0	0	0	0	1	1	0	1	1	1	0	3	1	0	2	0	0	2	1	0	3	1	1	0	0	0
*Ectatomma tuberculatum**	1	-	1	1	1	0	3	2	2	1	0	1	1	2	1	2	1	1	2	1	0	1	0	1	2	1	1	0	0	0
Ectatomma tuberculatum	1	-	1	1	1	0	3	2	2	1	0	1	1	2	1	2	1	1	2	1	0	1	0	1	2	1	1	0	0	1
*Camponotus sericeiventris**	2	-	1	0	0	1	0	0	0	1	0	1	0	0	0	3	1	0	1	0	0	1	1	0	0	1	1	1	1	0
Camponotus sericeiventris	2	-	1	0	0	1	0	0	0	1	0	1	0	0	0	3	1	0	1	0	0	1	1	0	0	1	1	1	2	0
*Camponotus sp 1**	2	-	1	1	0	1	0	0	0	0	1	1	0	0	0	3	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Camponotus sp 1	2	-	1	1	0	1	0	0	0	0	1	1	0	0	0	3	0	0	0	0	0	1	1	0	0	1	0	0	0	0
*Camponotus sp 2**	2	-	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Camponotus sp 2	2	-	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
*Camponotus sp 3**	2	-	1	0	0	2	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Camponotus sp 3	2	-	1	0	0	1	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	1	1	0	0	1	0	0	0	0
*Camponotus sp 4**	2	-	1	1	0	2	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Camponotus sp 4	2	-	1	1	0	2	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0
*Camponotus sp 5**	2	-	1	0	0	2	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Camponotus sp 5	2	-	1	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0
*Camponotus sp 6**	2	-	1	0	0	3	0	0	0	0	1	1	0	0	0	2	1	0	0	0	0	1	1	0	0	0	0	0	0	0
Camponotus sp 6	2	-	1	0	0	3	0	0	0	0	1	1	0	0	0	2	1	0	0	0	0	1	1	0	0	0	0	0	0	0
*Camponotus sp 7**	2	-	1	1	0	3	0	0	0	0	0	1	0	0	0	2	1	0	0	0	0	1	1	0	0	0	0	0	0	0
Camponotus sp 7	2	-	1	1	0	1	0	0	0	0	0	1	0	0	0	2	1	0	0	0	0	1	1	0	0	0	0	0	0	0
*Camponotus sp 8**	2	-	1	0	0	2	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	1	1	0	0	1	0	0	0	0
Camponotus sp 8	2	-	1	0	0	2	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	1	1	0	0	1	0	0	0	0
*Cephalotes maculatus**	2	-	1	0	0	1	1	0	0	0	1	1	1	2	0	1	0	3	1	0	1	1	?	1	1	1	0	0	0	0
Cephalotes maculatus	2	-	1	0	0	1	1	0	0	0	1	1	1	2	2	1	0	3	1	0	1	1	?	1	1	1	0	0	0	0
*Cephalotes basalis**	2	-	1	0	0	1	1	0	0	0	1	1	1	2	3	1	0	3	1	0	1	1	?	1	1	1	0	0	0	0
Cephalotes basalis	2	-	1	0	0	1	1	0	0	0	1	1	1	2	3	1	0	3	1	0	1	1	?	1	1	1	0	0	0	0
*Paraponera clavata**	0	1	1	1	0	0	1	1	2	1	0	1	1	1	1	3	1	1	2	1	0	1	0	1	1	0	0	0	0	0
Paraponera clavata	0	1	1	1	0	0	1	1	2	1	0	1	1	1	1	1	1	1	2	1	0	1	0	1	1	0	0	0	0	0
*Odontomachus hastatus**	0	1	1	0	0	0	2	1	2	1	0	1	1	1	1	2	1	1	0	0	0	0	0	1	2	1	0	0	0	2
Odontomachus hastatus	0	1	1	0	0	0	2	1	2	1	0	1	1	1	1	1	1	1	0	0	0	0	0	1	2	1	0	0	0	2
*Pachycondyla carinulata**	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	3	1	1	0	0	0	1	0	1	2	0	1	0	0	2
Pachycondyla carinulata	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	3	1	1	0	0	0	1	0	1	2	0	1	0	0	2
*Pachycondyla inversa**	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	3	1	1	0	0	0	1	0	1	2	0	1	0	0	2
Pachycondyla inversa	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	3	1	1	0	0	0	1	0	1	2	0	1	0	0	2
*Pachycondyla crenata**	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	2	1	1	0	0	0	1	0	1	2	0	1	0	0	2
Pachycondyla crenata	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	2	1	1	0	0	0	1	0	1	2	0	1	0	0	2
*Pachycondyla sp 1**	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	3	1	1	0	0	0	0	0	1	2	0	1	0	0	2
Pachycondyla sp 1	1	-	1	1	0	0	2	0	2	1	0	1	1	1	1	3	1	1	0	0	0	0	0	1	2	0	1	0	0	2
*Platythyrea pilosula**	1	-	1	0	0	0	0	0	2	1	0	1	1	1	1	3	1	1	0	0	0	0	0	1	2	0	1	0	0	2
Platythyrea angusta	1	-	1	0	0	0	0	0	2	1	0	1	1	1	1	3	1	1	0	0	0	0	0	1	2	0	1	0	0	2
Dasymutilla occidentalis	0	0	0	-	0	-	1	0	0	1	1	1	-	0	2	-	1	0	0	1	0	0	-	-	0	0	0	0	0	0
Atomacera debilis	0	0	0	1	0	-	0	0	0	1	0	0	-	0	0	-	-	1	0	-	1	-	-	-	0	-	0	0	0	2

Appendix 3. (*continued*)

					65					70					75					80					85					90					95				
										*	*															*	*			*									
	U				U					U	O							O							O			U			O	O		U	U	U	U		
*Dolichoderus sp 1**	0	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0	2	1	0	0	0	0	0	2	0	0	0	0	1	2	1	1	0	0	0	0	0	1
Dolichoderus sp 1	0	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0	2	1	0	0	0	0	0	2	0	0	0	0	1	2	1	1	0	0	0	0	0	1
*D. superaculus**	2	1	1	2	1	0	1	0	0	0	0	0	1	1	1	0	2	1	0	0	0	0	0	2	0	0	0	0	1	2	1	1	0	0	0	0	0	1
D. superaculus	2	1	1	2	1	0	1	0	0	0	0	0	1	1	1	0	2	1	0	0	0	0	0	2	0	0	0	0	1	2	1	1	0	0	0	0	0	1
*Dolichoderus sp 2**	0	1	1	2	0	0	1	0	0	0	0	0	0	1	1	0	2	1	0	0	0	0	0	2	0	0	0	0	1	2	1	1	0	0	0	0	0	1
Dolichoderus sp 2	0	1	1	2	0	0	1	0	0	0	0	0	0	1	1	0	2	1	0	0	0	0	0	2	0	0	0	0	1	2	1	1	0	0	0	0	0	1
*Ectatomma tuberculatum**	1	0	1	2	1	0	1	0	0	0	0	1	1	1	0	1	0	1	0	0	1	1	0	1	1	0	0	0	0	1	1	1	3	1	1	1	0	1
Ectatomma tuberculatum	1	0	1	2	1	0	1	0	0	0	0	1	1	1	0	1	0	1	0	0	1	1	0	1	1	0	0	0	0	1	1	1	3	1	2	2	0	1
*Camponotus sericeiventris**	1	0	0	0	2	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	0
Camponotus sericeiventris	1	0	0	0	2	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	0
*Camponotus sp 1**	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	1	1
Camponotus sp 1	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	0	0
*Camponotus sp 2**	1	0	0	0	0	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	1
Camponotus sp 2	1	0	0	0	0	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	1
*Camponotus sp 3**	2	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	1
Camponotus sp 3	2	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	1
*Camponotus sp 4**	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	1	1
Camponotus sp 4	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	1	0
*Camponotus sp 5**	2	0	0	0	2	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	0
Camponotus sp 5	2	0	0	0	2	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	1
*Camponotus sp 6**	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	1	0
Camponotus sp 6	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	1	0
*Camponotus sp 7**	1	0	0	0	0	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	0	1
Camponotus sp 7	1	0	0	0	0	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	0	1
*Camponotus sp 8**	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	1
Camponotus sp 8	1	0	0	0	1	0	1	1	1	0	0	0	1	1	1	0	1	1	0	0	0	0	1	2	0	0	0	0	0	2	0	1	0	0	0	0	0	1
*Cephalotes maculatus**	0	0	0	0	0	0	0	-	0	0	0	0	0	1	1	0	2	1	1	1	1	1	0	2	0	0	0	0	0	1	1	1	1	0	0	0	0	0
Cephalotes maculatus	0	0	0	0	0	0	0	-	0	0	0	0	0	1	1	0	2	1	1	1	1	1	0	2	0	0	0	0	0	1	1	1	1	0	0	0	1	0
*Cephalotes basalis**	0	0	0	0	0	0	0	-	0	0	0	0	0	1	1	0	2	1	1	1	1	1	0	2	0	0	0	0	0	1	1	1	1	0	0	0	0	0
Cephalotes basalis	0	0	0	0	0	0	0	-	0	0	0	0	0	1	1	0	2	1	1	1	1	1	0	2	0	0	0	0	0	1	1	1	1	0	0	0	0	0
*Paraponera clavata**	1	0	0	0	1	0	1	1	2	0	0	0	1	1	1	1	0	1	0	0	1	1	0	1	1	0	0	1	0	0	1	1	3	2	0	0	0	1
Paraponera clavata	1	0	0	0	1	0	1	1	2	0	0	0	1	1	1	1	0	1	0	0	1	1	0	1	1	0	0	1	0	0	1	1	3	2	0	0	0	1
*Odontomachus hastatus**	1	0	0	0	1	0	1	1	2	1	3	0	1	1	0	0	0	1	0	0	1	1	0	0	1	1	1	1	0	1	0	0	-	0	0	0	1	1
Odontomachus hastatus	1	0	0	0	1	0	1	1	2	1	3	0	1	1	0	0	0	1	0	0	1	1	0	0	1	1	2	1	0	1	0	0	-	0	0	0	1	1
*Pachycondyla carinulata**	1	0	0	0	1	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	-	0	0	0	1	1
Pachycondyla carinulata	1	0	0	0	2	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	-	0	0	0	0	1
*Pachycondyla inversa**	1	0	0	0	1	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	2	1	2	0	0	0	0	1
Pachycondyla inversa	1	0	0	0	1	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	2	1	2	0	0	0	0	1
*Pachycondyla crenata**	1	0	0	0	0	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	-	0	0	0	0	1
Pachycondyla crenata	1	0	0	0	1	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	1	0	-	0	0	0	0	1
*Pachycondyla sp 1**	1	0	0	0	0	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	1	1	2	0	0	0	0	1
Pachycondyla sp 1	1	0	0	0	0	0	1	1	2	0	2	0	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	1	1	1	2	0	0	0	0	1
*Platythyrea pilosula**	0	0	0	0	2	0	1	1	2	0	0	0	1	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1
Platythyrea angusta	0	0	0	0	2	0	1	1	2	0	0	0	1	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1
Dasymutilla occidentalis	2	0	0	0	1	1	1	1	1	2	1	-	1	1	1	0	1	1	1	-	1	-	-	0	0	0	0	0	-	2	1	-	-	0	0	0	-	-
Atomacera debilis	0	-	-	0	0	0	1	0	0	-	0	-	-	0	1	0	1	0	0	-	0	-	-	-	-	0	0	0	-	-	-	-	-	0	0	0	-	-

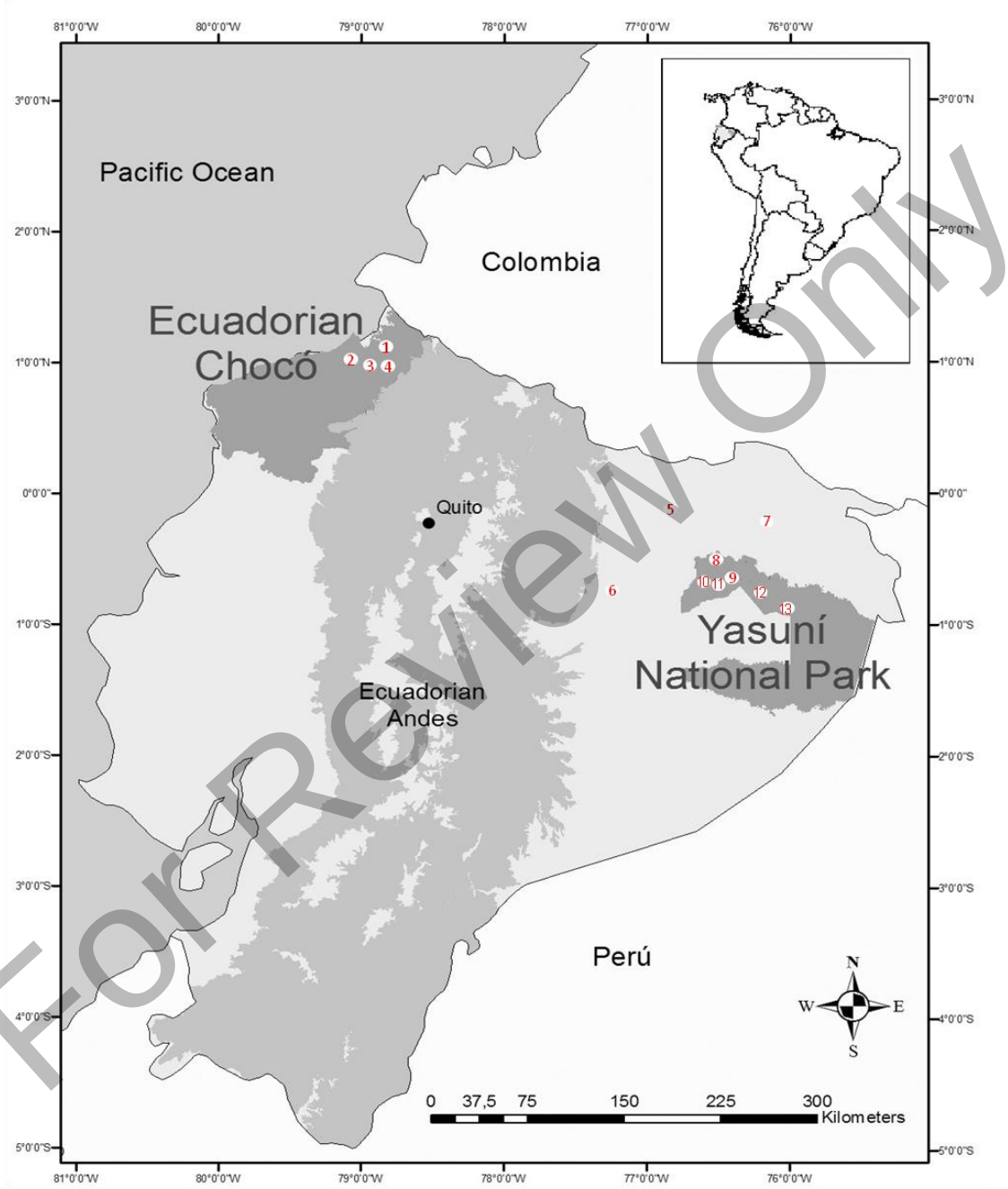
Appendix 4. Main morphological differences between ant species from Chocó and Amazonia found during the present study. Character numbers refer to Appendix 2. Character descriptions are detailed under results, except for the genera *Cephalotes* and *Plyatythyrea*.

	Species	Character	Chocó	Amazonia
Formicinae	*Camponotus sericeiventris*	Mesosoma: width of the mesosomal dorsal carinal edge (Char. 59)	does not vary accros its length	varies accros its length
	Camponotus sp. 1	Entire body: coloration pattern; surface type (Chars. 97, 98)	concolored: black head, rest of body brownish. Shining body surface	single colored: brownish. Opaque body surface
	Camponotus sp. 2	no discernible morphological dissimilarities		
	Camponotus sp. 3	Head: impressed line between antennal sockets (Char. 36)	clearly visible	poorly visible
	Camponotus sp. 4	Entire body: surface type (Char. 98)	relatively shining	opaque
	Camponotus sp. 5	Mesosoma: color (Char. 46)	black	light brown
		Entire body: surface type (Char. 98)	opaque	shining
	Camponotus sp. 6	no discernible morphological dissimilarities		
	Camponotus sp. 7	Head: impressed line between antennal sockets (Char. 36)	clearly visible and protruding	poorly visible
	Camponotus sp. 8	no discernible morphological dissimilarities		
Myrmicinae	*Cephalotes basalis*	Mesosoma: dorso-anterior propodeal spines	two well defined	one blunt spine
		Head: supraocular carina	protruding from top of the eye and forming a wave-like shape	a simple ridge, not protruding from top of the eye
	Cephalotes maculatus	Mesosoma: color (Char. 46)	light brown	dark brown
Dolichoderinae	*Dolichoderus superaculus*	Head: first segment of funiculus (Char. 31)	longer than the second segment	around the same size compared to the second segment
	Dolichoderus sp. 1	Mesosoma: color (Char. 46)	redish black	black
	Dolichoderus sp. 2	no discernible morphological dissimilarities		

Appendix 4. (*Continued*)

	Species	Character	Chocó	Amazonia
Ectatomminae	*Ectatomma tuberculatum*	Mesosoma: sutural line dividing anepisternum from katepisternum (Char. 60)	absent	poorly visible
		Metasoma: arrangement and amount of striae in posterior face of petiole (Char. 95)	few striae present; striae-free area is discernible in the center of the posterior petiolar face	more striae present; no striae-free area is discernible
		Orientation of striae at inner dorsal region on third abdonimal segment (Char. 96)	running vertically	running horizontally
Ponerinae	*Odontomachus hastatus*	Mesosoma: color (Char. 46)	light Brown	dark brown
		Metasoma: orientation of external part of petiolar spine in pedunculated petiole (Char. 87)	erect	curved backwards
		Metasoma: mid frontal face of petiole	with a slight concavity	not cancave, instead slightly convex
	Pachycondyla carinulata	Mesosoma: cuticular shape of metapleuron (Char. 65)	maily striated	mainly with foveae of different size
		Entire body: coloration pattern (Char. 97)	concolored	single colored
	Pachycondyla crenata	Mesosoma: cuticular shape of metapleuron (Char. 65)	smooth with no rugosities or foveae	mainly striated
	Pachycondyla inversa	no discernible morphological dissimilarities		
	Pachycondyla sp.1	no discernible morphological dissimilarities		
	Platythyrea	Mesosoma: fore femur width	*Platythyrea pilosula*: 0.40 mm (average)	*Platythyrea angusta*: 0.50 mm (average)
		Mesosoma: fore femur length	*Platythyrea pilosula*: 1.26 (average)	*Platythyrea angusta*: 1.03 (average)
Paraponerinae	*Paraponera clavata*	Head: hairy pleurostomal area (Char. 27)	standing setae length ca. 0.19 mm in average and more abundant than in species from Amazonia	standing setae length ca. 0.08 mm in average
		Mesosoma: color (Char. 46)	black	redish black

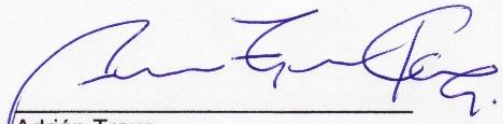
Appendix 5. Map of Ecuador showing collection sites of the ant specimens analyzed in this study. Most sampling sites in Amazonia are located in the Yasuní National Park. (Map authorship: Luis Espinosa)



Chocó		Amazonia			
1	Pajonal	5	Bermejo	9	Guiyero, L. Guaorani
2	La Tabla	6	Jatun Sacha	10	Guiyero, L. Tigre
3	C. Vicente	7	Cuyabeno	11	Guiyero, B. Nambay
4	S. Bravo	8	Guiyero	12	Onkonegare
				13	Tiputini

8. Statement of Originality

Unless otherwise acknowledged, I confirm that no part of this study has been published before. The questions and the entire manuscript were planned and elaborated by the autor.



Adrián Troya
Munich, September 2012

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Titel der Arbeit: ANT SPECIATION IN THE TROPICS OF SOUTH AMERICA

Arbeitsgruppe, in der die Arbeit angefertigt wurde:

Betreuer/in der Arbeit: PROF. DR. GERHARD HASZPRUNAR

Unterschrift Betreuer/in:

Weitere/r Prüfende/r der Arbeit: DR. STEPHAN HUTTER

Datum des Beginns der Arbeit: 1. MÄRZ. 2012

Datum der Abgabe der Arbeit: 31. AUGUST. 2012

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