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Molecular phylogenetics of ponerine ants (Hymenoptera: Formicidae: Ponerinae)

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Abstract

Recent molecular phylogenetic studies of ants (Hymenoptera: Formicidae) have revolutionized our understanding of how these ecologically dominant organisms diversified, but detailed phylogenies are lacking for most major ant subfamilies. I report the results of the first detailed phylogenetic study of the ant subfamily Ponerinae, a diverse cosmopolitan lineage whose properties make it an attractive model system for investigating social and ecological evolution in ants. Molecular sequence data were obtained from four nuclear genes (*wingless*, *long-wavelength rhodopsin*, *rudimentary* [CAD], 28S rDNA; total of ~3.3 kb) for 86 ponerine taxa, representing all three ponerine tribes, 22 of the 28 currently recognized genera, and 14 of the 18 informal subgenera of *Pachycondyla*, a heterogeneous grouping whose monophyly is doubtful on morphological grounds. Phylogenetic reconstructions using maximum likelihood and Bayesian inference support the monophyly of Ponerinae and tribe Platythyreini, but fail to support the monophyly of the large tribe Ponerini due to its inclusion of the unusual genus *Thaumatomyrmex*. *Pachycondyla* is inferred to be broadly non-monophyletic. Numerous novel generic and suprageneric relationships are inferred within Ponerini, which was found to consist of four major multi-generic clades (the *Ponera*, *Pachycondyla*, *Plectroctena* and *Odontomachus* genus groups) plus the single genera *Hypoponera* and *Harpegnathos*. Uncertainty remains in some regions of the phylogeny, including at the base of Ponerini, possibly reflecting rapid radiation. Divergence dating using a Bayesian relaxed clock method estimates an origin for stem Ponerinae in the upper Cretaceous, a major burst of diversification near the K/T boundary, and a rich and continual history of diversification during the Cenozoic. These results fail to support the predictions of the "dynastic-succession hypothesis" previously developed to explain the high species diversity of Ponerinae. Though model-based reconstructions of historical biogeography and trait evolution were not attempted in this study, the phylogeny suggests that ponerine evolution was marked by regionalized radiations and frequent faunal exchange between major biogeographic provinces. The reported results also imply multiple origins of cryptobiotic foraging, mass raiding behavior, and gamergate reproduction within Ponerinae, highlighting the value of the subfamily as a model for studying the incipient evolution of these and other ecological and behavioral traits.

Key words: relationships, radiations, systematics, phylogenetic inference, divergence dating

Introduction

A common theme in the study of terrestrial ecosystems is the ecological importance of ants (Hymenoptera: Formicidae). Though they make up only a small fraction of total insect species diversity (with roughly 12,500 described species; Agosti and Johnson, 2009), ants have few rivals among animals in their abundance, total biomass, range of ecological interactions, and influence on ecosystem-level processes (Fittkau and Klinge, 1973; Wilson, 1990; Hölldobler and Wilson, 1990; Agosti *et al.*, 2000; Kaspari *et al.*, 2000; Wilson and Hölldobler, 2005). Much of the ecological success of ants can be attributed to their advanced sociality, which provides greater efficiency and the exploitation of otherwise unavailable niches (Wilson, 1971; Oster and Wilson, 1978; Hölldobler and Wilson, 1990). Recent molecular phylogenetic studies of ants (*e.g.*, Brady, 2003; Ward and Brady, 2003; Saux *et al.*, 2004; Ward and Downie, 2005; Moreau *et al.*, 2006; Brady *et al.*, 2006; Ouellette *et al.*, 2006; Rabeling *et al.*, 2008; Ward *et al.*, 2010) have revolutionized our understanding of the basic course of ant evolution. Ward *et al.* (2010) recently published the first detailed genus-level phylogeny of one of the "big four" ant subfamilies, the Dolichoderinae, but comprehensive phylogenies are still lacking for Myrmicinae, Formicinae, and Ponerinae.

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Detailed phylogenies for these groups would enable more robust investigations of the selective forces driving ant social evolution and the historic rise of ants to ecological dominance.

I report results of the first detailed molecular phylogenetic study of the ant subfamily Ponerinae (*sensu* Bolton, 2003). Ponerinae is the third most species-rich ant subfamily with over 1,100 described extant species, and exhibits a primarily pantropical geographic distribution (Bolton *et al.*, 2006; current species count from Antweb, 2012). Unlike other ant lineages of comparable age and diversity, ponerines generally display a suite of behavioral and ecological traits that are considered ancestral within ants, including small colony size, a monomorphic worker caste, poor morphological differentiation between workers and queens, and generalized solitary predaceous foraging (Peeters, 1997; Wilson and Hölldobler, 2005). From this general condition, ponerines have evolved a substantial diversity of social organizations and foraging behaviors. To a surprising degree, Ponerinae resembles a condensed microcosm of the morphological, ecological and social diversity within Formicidae (although some repeated trends in ant evolution, such as social parasitism and the tending of honeydew-secreting insects, are rare or absent in Ponerinae; Hölldobler and Wilson, 1990). Ponerines therefore provide an almost unparalleled opportunity to study the incipient evolution of many traits that have arisen repeatedly during the course of ant evolution.

Ponerinae has never been the subject of a thorough phylogenetic analysis, preventing broad evolutionarily-informed studies of ponerine social or ecological evolution. Most higher-level relationships within the subfamily are therefore poorly understood. Ponerinae has also never received a modern phylogenetically-based taxonomic revision, and the current tribal and generic classification remains untested. Ponerinae is currently divided into three tribes (Fig. 1): the monogeneric tribes Platythyreini (*Platythyrea*, 39 extant species) and Thaumatomyrmecini (*Thaumatomyrmex*, 12 extant species), and the diverse tribe Ponerini (26 genera, 1,051 extant species). The monophyly of Platythyreini is supported by morphological synapomorphies (Bolton, 2003), but morphological evidence is ambiguous regarding the reciprocal monophyly of Thaumatomyrmecini and Ponerini. A small number of ponerine taxa have been included in other phylogenetic studies of ants, and these have supported the separate tribal status of Platythyreini (Moreau *et al.*, 2006; Brady *et al.*, 2006). The only previous phylogenetic study to include Thaumatomyrmecini in its sampling (Brady *et al.*, 2006) placed it within a non-monophyletic Ponerini.

From both taxonomic and evolutionary perspectives, a more pressing problem is the status of the ponerine genus *Pachycondyla*. As presently defined (Bolton *et al.*, 2006), *Pachycondyla* is a large assemblage of 237 described extant species and is the end result of numerous generic synonymizations. These synonymizations were mostly the result of work by W. L. Brown, though his justifications for these actions were never published. Brown's conception of *Pachycondyla* was based on symplesiomorphies (W. L. Brown, unpublished m.s.), and the lack of synapomorphies for the genus, its substantial heterogeneity, and the obvious close relationships of some of its members to other genera cast significant doubt on its monophyly.

The principal goals of this study were (1) to test the current tribal and generic classification of Ponerinae, particularly the monophyly of Ponerini and *Pachycondyla*; (2) to infer higher relationships within the subfamily; (3) to estimate the time scale of ponerine evolution; and (4) to provide a historical framework for future studies of ponerine biogeography and morphological, ecological and social evolution.

Materials and methods

Taxon Sampling. This study follows the currently accepted taxonomic framework for Ponerinae (Bolton, 2003; Bolton *et al.*, 2006; Fisher, 2006; Bolton and Fisher, 2008C), though for ease of communication I treat junior synonyms of *Pachycondyla* as informal subgenera; this should not be considered a formal designation of subgeneric status. A revised classification of ponerine tribes and genera, based partially on the results reported here, will be published in a companion paper (C. Schmidt and S. Shattuck, in prep).

Molecular sequence data were generated for 80 ponerine taxa (the "Core Ingroup" taxon set), representing all three ponerine tribes, 22 of the 28 currently recognized genera, and 14 of the 18 "subgenera" of *Pachycondyla.* Several exceedingly rare genera could not be sampled for this study. Taxa were selected for sequencing based on specimen availability, taxonomic significance (type species were chosen when possible), and to maximize geographic breadth and morphological and behavioral diversity. Genera with high species or behavioral diversity, high likelihood of non-monophyly, or critical phylogenetic importance were sampled more heavily. To supplement

this taxon sampling, I integrated data for all 15 ponerine species included by Brady *et al.* (2006) in their molecular phylogeny of Formicidae. Nine of these species overlapped my own sampling, while the remaining six species (the "Additional Ingroup" taxon set) were additions to my sampling but did not represent any additional genera.

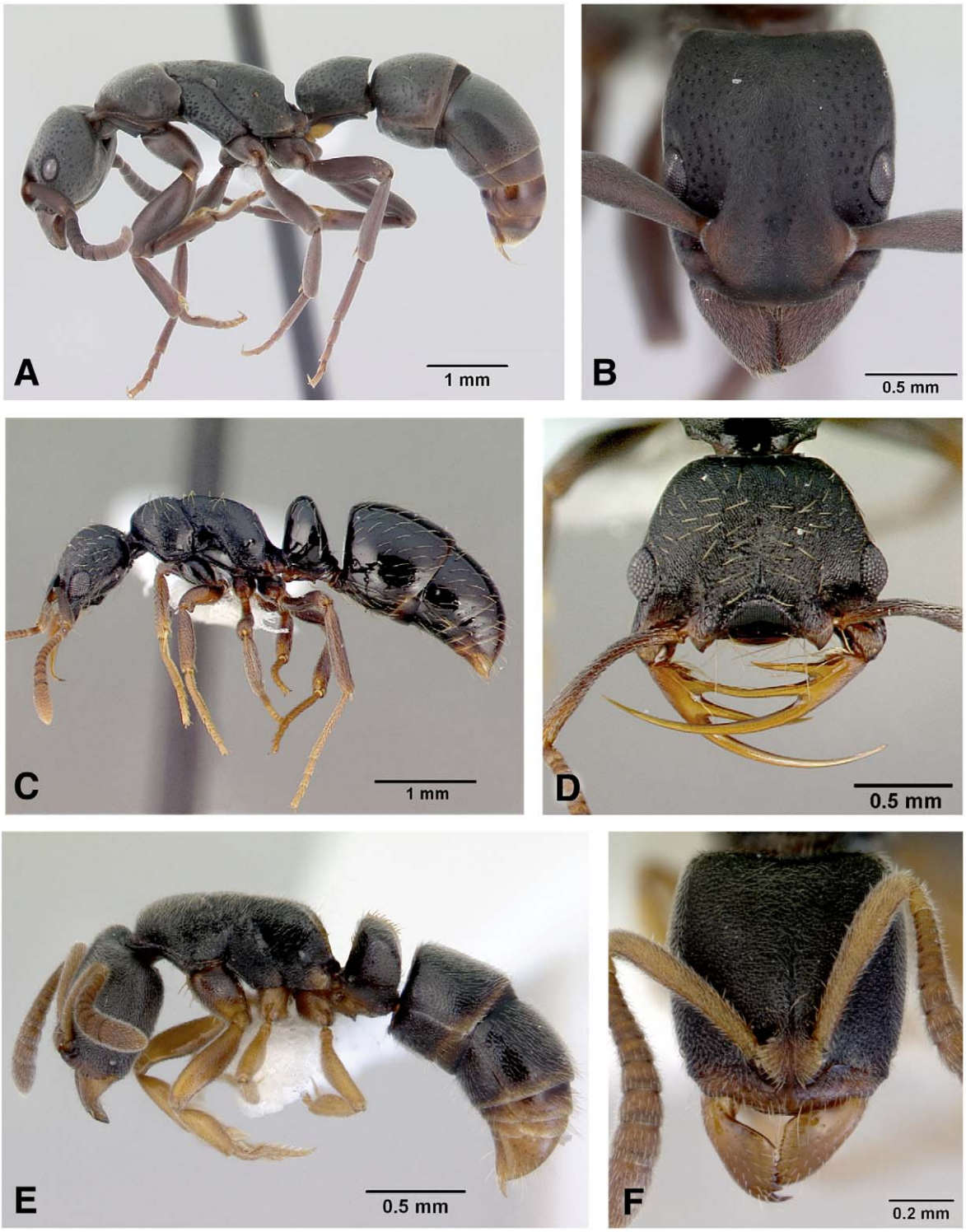


FIGURE 1. Lateral and frontal views of a member of each ponerine tribe: (A-B) *Platythyrea pilosula* (Platythyreini); (C-D) *Thaumatomyrmex* cf. *cochlearis* (Thaumatomyrmecini), (E-F) *Ponera selenophora* (Ponerini). Photographs courtesy of April Nobile and http://www.antweb.org; provided through a Creative Commons Attribution-Non Commercial 3.0 Unported license: http://creativecommons.org/licenses/by-nc/3.0/.

Outgroup taxa were drawn mainly from Brady *et al.* (2006) (the "Core Outgroup" taxon set). From their extensive data set I selected 44 ant species that represented all extant ant subfamilies described to that time. These species were chosen to maximize phylogenetic breadth and informative fossil constraints in the dating analyses. Special emphasis was given to the poneroid subfamilies (*sensu* Brady *et al.*, 2006) to provide improved phylogenetic signal at the base of Ponerinae. In addition to these ant outgroups, I included several non-ant hymenopteran outgroups sequenced by Brady *et al.* (2006) to root Formicidae and improve robustness of the dating analyses. These outgroups included members of the vespoid families Bradynobaenidae, Mutillidae, Pompilidae, and Tiphiidae, the apoid families Apidae and Sphecidae, and the chrysidoid family Bethylidae. I also included published sequence data for *Martialis heureka* Rabeling & Verhaagh (Martialinae), a recently described taxon that may be sister to all other extant Formicidae (Rabeling *et al.*, 2008; but see Kück *et al.*, 2011). Taxon and gene sampling for this study are summarized in Table 1. Full lists of taxa, including voucher numbers and GenBank accession numbers, are provided in Supp. Table S1 (ingroup) and Supp. Table S2 (outgroup).

TABLE 1. Summary of taxon and gene sampling. Percentages are the proportion of taxa sequenced for each gene fragment. "All Genes" is the percent completion of the matrix for that taxon set across all genes.

		Core Genes				Secondary Genes			
Taxon Set	Taxa	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2	All Genes
Core Ingroup[1]	80	100%	96%	98%	85%	11%	11%	11%	59%
Additional Ingroup[2]	6	100%	100%	0%	100%	100%	100%	100%	86%
Core Outgroup[3]	51	100%	100%	12%	100%	100%	100%	100%	87%
Martialis heureka[4]	1	0%	0%	0%	100%	0%	0%	100%	29%
All Taxa	138	99%	97%	60%	92%	48%	48%	49%	70%

1 Most core gene data are from this study (see section 2.2 for details); secondary genes are from Brady et al (2006).
2 Data from Brady *et al.* (2006).
3 Most data from Brady *et al.* (2006) except CAD for *Apis mellifera* (GenBank) and five ant taxa (Ward *et al.*, 2010).
4 Data from Rabeling *et al.* (2008).

Gene sampling and DNA sequencing. Fragments of four nuclear genes (the "core genes" of this study) were sequenced for the Core Ingroup taxa: *wingless* (wg), *long-wavelength rhodopsin* (LW Rh), *rudimentary* (CAD), and the nuclear large subunit (28S) ribosomal RNA gene. Sequence data were available from GenBank for a small number of ponerine taxa utilized in previous studies; these data were used to fill sequencing gaps in the data matrix when possible. Of the 304 sequences obtained for the core genes in the Core Ingroup taxa, 31 were previously published (Ohnishi *et al.*, 2003; Brady *et al.*, 2006; Moreau *et al.*, 2006; Spagna *et al.*, 2008); the remaining 273 sequences were generated for this study. After excluding hypervariable sections of 28S and introns of LW Rh and CAD, the concatenated aligned data matrix for these genes was about 3.3 kb in length (with 1,514 parsimony-informative sites across all taxa and 1,234 parsimony-informative sites within Ponerinae) and was about 95% complete for the core ingroup taxa. Not all sequences were complete; several CAD sequences, in particular, were only partially obtained due to recalcitrant amplification in some taxa.

Ponerine and outgroup taxa integrated from external sources had a somewhat different gene sampling. For the taxa taken from Brady *et al.* (2006), sequences were included for wg, LW Rh, 28S, *abdominal-A* (abdA), EF1αF1, and EF1αF2 (the latter three constitute the "secondary genes" of this study). I also included 28S and EF1αF2 sequences for *Martialis heureka* (Rabeling *et al.*, 2008) and CAD sequences for *Apis mellifera* Linnaeus (GenBank acc. DQ067178) and several ant outgroup taxa from Ward *et al.* (2010). The final concatenated matrix was nearly 5 kb in length (with 2,055 parsimony-informative sites among all taxa and 1,521 parsimony-informative sites within Ponerinae) and was about 70% complete across all included taxa. Sequence characteristics of each sampled gene are given in Table 2.

Genomic DNA was extracted from one or two legs of a single adult female specimen (usually a worker) of each taxon using the DNEasy Tissue Kit (Qiagen Inc., Valencia, California, USA), and diluted to a total volume of 100 µl. For particularly large specimens (*e.g.*, *Dinoponera australis* Emery, *Streblognathus peetersi* Robertson) only a portion of a single leg was used. The remainder of the specimen was retained in 100% EtOH until verification of extraction success, then mounted to facilitate identification and to act as a voucher. All mounted

specimen and DNA vouchers will be deposited with the California Academy of Sciences in San Francisco, California; CASENT voucher numbers are given in Supp. Table S1.

TABLE 2. Sequence characteristics of genes utilized in this study. Variable and parsimony-informative ("Pars.-Inf.") sites are given for all taxa and for ingroup taxa (all Ponerinae) alone.

		All Taxa		Ingroup Only	
Gene	Total Sites	Variable	Pars.-Inf.	Variable	Pars.-Inf.
Wg	451	281	234	230	194
LW Rh	467	273	250	233	214
CAD	1,522	828	749	805	728
28S	899	436	281	191	98
AbdA	630	267	220	141	91
EF1αF1	359	138	130	89	45
EF1αF2	517	204	191	175	151
All Genes	4,845	2,427	2,055	1,864	1,521

Genes were amplified using standard PCR techniques. Reaction mixtures consisted of 1 µL of DNA template, 1 µL of dNTP mix (Eppendorf AG, Hamburg, Germany), 1 µL of each primer (5 mM), 0.1 µL of Hotmaster Taq (Eppendorf AG) or Ex Taq (Takara Bio Inc., Shiga, Japan), 2.5 µL of 10x buffer, and 18.4 µL of PCR water, for a total reaction volume of 25 µL. PCR programs utilized for wg, LW Rh, and 28S entailed an initial melting at 94° C (2 min.), 40 cycles of 94° C (20 s.), 50-54° C (20 s.), and 65° C (HotMaster) or 72° (ExTaq), and a final extension at 65° or 72° (5 min.). Amplification of CAD was found to be most successful with a "touch-down" program of an initial melting at 94° C (2 min.), 10 cycles of 94° (30 s.), 60° C (30 s., decreasing by 1° C/cycle), and 65°/72° C (90 s.), followed by 30 cycles with identical settings but an annealing temperature of 50° C, and a final extension of 65°/72° C (5 min.). The latter PCR program also proved helpful with the other sampled genes in difficult cases. Cleaning, quantification and Sanger sequencing were performed at the GATC sequencing facility of the University of Arizona.

Primers utilized in this study are listed in Table 3. Amplification of wg was usually performed using the primer combination Wg578F/Wg1032R; Wg550F/Wg1032R was used for difficult cases, sometimes nested with Wg578F/Wg1032R, and WgAbRZ was sometimes substituted for Wg1032R. Amplification of LW Rh usually entailed the combination LR134F/LR639ER; for difficult cases LR140F/LR639ER was used, sometimes nested with LR140F/LR505R2 and LR395F/LR639ER. Amplification of CAD was complicated by the presence of numerous long introns, which necessitated the use of three overlapping amplicons employing the following primer combinations: (1) CD847F/CD1465R nested with CD847F/CD1459R; (2) CD1267F/CD1879R nested with CD1421F/CD1879R; and (3) CD1679F/CD2362R nested with CD1821F/CD2362R. In some cases two or even all three CAD fragments were amplified as a single amplicon and then re-amplified with the nested primer pairs given above.

Sequences were aligned using the pairwise alignment tool in MacClade 4.08 (Maddison and Maddison, 2005) and adjusted manually. Ambiguously aligned regions of 28S, and introns in LWR and CAD, were excluded prior to phylogenetic analysis.

Phylogenetic inference. Molecular phylogenies were inferred using three methods: partitioned Bayesian analyses in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003; Altekar *et al.*, 2004), partitioned relaxed clock Bayesian analyses in BEAST v.1.4.8 (Drummond *et al.*, 2006; Drummond and Rambaut, 2007), and unpartitioned maximum likelihood analyses in RAxML v7.0.4 (Stamatakis, 2006; while RAxML allows for data partitioning, unpartitioned analyses were conducted to provide a less model-intensive and thereby more independent assessment of the data vis-à-vis the Bayesian analyses).

Phylogenetic relationships within Ponerinae were inferred from the entire concatenated data matrix; in addition, numerous supplementary analyses were performed to explore various aspects of the data and to identify areas of uncertainty in the inferred phylogeny. By analyzing various subsets of the data (Table 4) I was able to assess the impact of missing data, the degree of discordance among sampled loci, the possibility of topological artifacts resulting from long branch attraction, and the influence of aberrant CAD sequences for the genus

Harpegnathos on the inferred phylogenies. All data subsets were analyzed with MrBayes and RAxML (including bootstrap estimation), and the PONERINI_ONLY matrix was also analyzed with BEAST.

TABLE 3. List of primers utilized in this study for both PCR and sequencing reactions. CAD primers are named according to the corresponding nucleotide positions in the *Apis mellifera* sequence (GenBank acc. DQ067178).

Gene	Primer	Sequence (5' to 3')	Source
Wg	Wg550F	ATGCGTCAGGARTGYAARTGYCAYGGYATGTC	Wild and Maddison (2008)
	Wg578F	TGCACNGTGAARACYTGCTGGATGCG	Ward and Downie (2005)
	Wg1032R	ACYTCGCAGCACCARTGGAA	Abouheif and Wray (2002)
	WgAbRZ	CACTTNACYTCRCARCACCARTG	Wild and Maddison (2008)
LW Rh	LR134F	ACMGTRGTDGACAAAGTKCCACC	Ward and Downie (2005)
	LR140F	GTWGACAAAGTKCCACCNGANATG	This study
	LR395F	AGGTGATYAATTGYTATTAYGARACGTGGGT	This study
	LR505R2	TCRTCRAATGCRATCATYGTCATYGWCCAAATGGAG	This study
	LR639ER	YTTACCGRTTCCATCCRAACA	Ward and Downie (2005)
28S	3318F	CCCCCTGAATTTAAGCATAT	Schmitz and Moritz (1994)
	4068R	TTGGTCCGTGTTTCAAGACGGG	Belshaw and Quicke (1997)
CAD	CD847F	ATGAATTACGGYAATCGCGGYCAYAAYCARCC	Adapted from Moulton and Wiegmann (2004)
	CD1267F	GARTTYGAYTATTCRGGSTCGCARGCG	This study
	CD1421F	AGGTAATACRATCRGARAGRCCDGACGG	This study
	CD1679F	TGGGTTATCCTGTTATGGCNCGYG	This study
	CD1821F	AGGYTGGAARGARGTVGARTAYGARGT	This study
	CD1459R	GCARTTDAGAGCGGTYTGYCCRCCRAAYGT	Adapted from Moulton and Wiegmann (2004)
	CD1465R	GCAATTAAGAGCRGTYTGYCCRCC	Adapted from Moulton and Wiegmann (2004)
	CD1879R	TGGATRCCGAGRGGATCGACRTTYTCCATRTTRCAYAC	This study
	CD2362R	GACCATCCTCAAAGCCTTYTGRAARGC	This study

ALL_DATA. The entire data matrix, consisting of all sampled taxa (138) and all genes, was analyzed to provide the baseline hypothesis of ponerine relationships. The final aligned and concatenated ALL_DATA matrix, and the consensus tree resulting from its analysis in MrBayes, have been deposited with TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13082).

CORE_DATA. To assess the effects of missing data and of the inclusion of outgroups on the inferred relationships within Ponerinae, I constructed a reduced data matrix consisting only of the Core Genes and most Core Ingroup taxa. The following Core Ingroup taxa were excluded because they had large proportions of missing data: *Platythyrea turneri* Forel, *Pachycondyla* (*Hagensia*) *havilandi* Forel, *Pachycondyla* (*Mesoponera*) *rubra* Smith, and *Ponera pennsylvanica* Buckley. In addition, the two sampled *Thaumatomyrmex* species (*T. atrox* Weber and *T.* sp. Bra1) were merged to form a single composite taxon due to their incomplete but mutually complementary gene sampling. The final data matrix consisted of 76 taxa.

PONERINI_ONLY. I explored the phylogenetic impact of including outgroups and *Platythyrea* on the inferred relationships within Ponerini (including the position of the root) by building a reduced data subset containing only members of Ponerini (80 taxa, all genes). The position of the root was free to vary in BEAST.

ALL_BUT_CAD. A substantial portion of the ALL_DATA matrix is composed of CAD (749 of 2,055 parsimony-informative sites), but this gene was not sampled for most outgroups. I therefore reanalyzed the ALL_DATA matrix with CAD excluded to evaluate the influence of CAD on the inferred phylogeny and determine whether it was obscuring an alternate topology that was better supported by other genes. This data set consisted of all 138 taxa.

TABLE 4. Summary of sequence characteristics, MCMC burnin and total chain lengths (MrBayes and BEAST) and final likelihood scores (RAxML) for data sets analysed in phylogenetic inference. Burnin and total chain lengths are in millions of cycles. "Coverage": Percent completion of the data matrix across all included taxa and genes. "-": Analysis not performed.

			Sequence Characteristics			MrBayes		BEAST		RAxML
Data Set	Taxa	Coverage	Total Sites	Variable	Pars.-Inf.	Burnin	Total	Burnin	Total	-lnL
ALL_DATA[1]	138	70%	4,845	2,427	2,059	14	27	-	-	94104.214
ALL_DATA[2]	138	70%	4,845	2,427	2,059	13	24	-	-	93580.384
ALL_DATA[3]	138	70%	4,845	2,427	2,059	25	50	-	-	93090.708
CORE_DATA[1]	76	97%	3,339	1,436	1,221	16	31	-	-	41286.347
CORE_DATA[3]	76	97%	3,339	1,436	1,221	-	-	-	-	40264.305
PONERINI_ONLY[1]	80	64%	4,845	1,754	1,407	9	16	62	73	45292.409
PONERINI_ONLY[2]	80	64%	4,845	1,754	1,407	17	33	95	100	44750.278
PONERINI_ONLY[3]	80	64%	4,845	1,754	1,407	6	11	95	100	44269.637
ALL_BUT_CAD	138	72%	3,323	1,599	1,310	33	65	-	-	65935.807
WG_LWR_28S	137	96%	1,817	990	766	5	10	-	-	40296.405
SEC_GENES	57	100%	1,506	609	541	5	10	-	-	24900.780
CAD	83	100%	1,522	828	749	4	9	-	-	27725.825
CAD_1nt/2nt	83	100%	1,014	339	268	5	10	-	-	8433.696
CAD_3nt	83	100%	508	489	481	25	49	-	-	18443.038

1 Entire CAD sequence for *Harpegnathos* included.
2 CAD 1nt/2nt for *Harpegnathos* excluded.
3 All of CAD sequence for *Harpegnathos* excluded.

WG_LWR_28S, SEC_GENES, CAD. To assess the degree of congruence among the sampled genes, I divided the entire matrix into three submatrices of approximately equal length: WG_LWR_28S (1,817 bp), SEC_GENES (1,505 bp), and CAD (1,522 bp). The WG_LWR_28S matrix consisted of Wg, LWR and 28S, and all taxa except *Martialis* (137 total), whereas the SEC_GENES matrix consisted of Abd-A, EF1αF1 and EF1αF2, and contained only the 57 taxa taken from Brady *et al.* (2006). CAD (83 taxa sampled) presented more of a challenge than the other genes and was analyzed in more detail. CAD sequences obtained for both species of *Harpegnathos* were unusual, with many amino acid changes at otherwise highly conserved sites. To assess the possibility that these sequences represented pseudogenes, paralogs, or the result of intense selection or drift, I analyzed the CAD data in the following variations: (1) all positions of CAD; (2) only first and second positions (1nt/2nt) of CAD; and (3) only third positions (3nt) of CAD. CAD analyses were rooted at *Apis mellifera*.

Given the uncertain reliability of the CAD sequences for *Harpegnathos* and the importance of *Harpegnathos* for the reconstruction of ponerine evolution, I analyzed variations of the ALL_DATA, CORE_DATA, and PONERINI_ONLY data matrices with: (1) all of CAD included for *Harpegnathos*; (2) first and second positions (1nt/2nt) of CAD excluded for *Harpegnathos*; and (3) all of CAD excluded for *Harpegnathos*.

For Bayesian analyses each protein-coding gene was partitioned by codon position (first and second positions combined into one partition, and third positions in a separate partition), with 28S assigned its own partition, yielding a total of 19 partitions in combined analyses of all genes. Substitution models were selected for each partition using the Akaike Information Criterion (AIC), which was estimated using MrModeltest v.2.2 (Nylander, 2004). Each partition was assigned an individual GTR+I+Γ model except third codon positions of CAD and EF1αF2, which were assigned individual HKY+I+Γ models. Base frequencies were unlinked across partitions, but topology and branch lengths were linked across partitions.

All MrBayes analyses were performed using default priors. Two independent analyses were run simultaneously, each with four chains and a temperature parameter of 0.23 (determined through trial and error to provide optimal chain mixing for these data). Parameters and trees were sampled every 1,000 generations. Runs

were allowed to continue until convergence was reached, as determined from plots of likelihoods and sampled parameter values in Tracer v1.4 (Rambaut and Drummond, 2007) and from the average standard deviation of split frequencies, which was calculated from the last 50% of sampled trees (analyses were terminated once this statistic fell below 0.01). Burnins consisted of at least the first 50% of generations, but were longer if model parameters were slow to converge. Total chain lengths and burnin lengths varied greatly between analyses (summarized in Table 5). Fully resolved majority rule consensus trees with mean branch lengths were calculated in MrBayes.

TABLE 5. Summary of MCMC burnin and total chain lengths (in millions of cycles) for phylogenetic dating analyses in BEAST.

Root Age (My)	Burnin	Total
185	20	34
155 [1]	18	28
155 [2]	14	24
155 [3]	83	93
145	25	35

1 Entire CAD sequence for *Harpegnathos* included.
2 CAD 1nt/2nt for *Harpegnathos* excluded.
3 All of CAD sequence for *Harpegnathos* excluded.

Analyses in BEAST were conducted using a relaxed molecular clock with uncorrelated log-normally-distributed branch rates (Drummond *et al.*, 2006) and the constant rate birth-death process for the prior distribution on node heights (Gernhard, 2008), with default priors. For some analyses the position of the root was specified by creating taxa blocks for the ingroup and outgroup, constraining the monophyly of each, and providing a starting tree containing the two clades each collapsed into a polytomy. A random coalescent starting tree, using default values for demographic parameters, was used for analyses in which BEAST was allowed to infer the root position. Convergence of likelihoods and model parameters was determined using Tracer. Most runs were terminated once these measures had been stable for at least 10 million generations, with preceding generations discarded as burnin. Total chain lengths and burnin lengths varied greatly between analyses (Table 5). Maximum clade credibility trees with mean node depths were calculated in TreeAnnotator v1.4.8 (Drummond and Rambaut, 2007).

Maximum likelihood analyses of unpartitioned data sets were conducted using the rapid bootstrapping estimation and subsequent fast ML optimization of RAxML (Stamatakis *et al.*, 2008). For each analysis 5,000 bootstrap replicates were employed, with the GTR+I+Γ model used for the final ML search.

Most consensus trees were rooted with *Pristocera* sp. (Chrysidoidea, Bethylidae), which was considered the deepest outgroup due to the apparent sister relationship between Chrysidoidea and the rest of Aculeata (as evidenced by morphological data: Brothers and Carpenter, 1993; Grimaldi and Engel, 2005). Certain BEAST analyses of limited taxon sets were rooted otherwise, as already described.

Phylogenetic hypothesis tests. Alternative hypotheses for relationships within Ponerini (including *Thaumatomyrmex*) were evaluated using Shimodaira-Hasegawa (SH) tests (Shimodaira and Hasegawa, 1999; Goldman *et al.*, 2000). Maximum likelihood analyses of the CORE_DATA matrix, with all of CAD excluded for *Harpegnathos*, were conducted in RAxML with and without topological constraints, using 20 random replicates and the GTR+I+Γ model. Constrained topologies were compared with the unconstrained topology using an SH test in PAUP* (Swofford, 2003), with 200,000 RELL bootstrap pseudoreplicates and parameters of the evolutionary model fixed at their maximum likelihood values from the unconstrained analyses. Specific hypotheses tested include the monophyly of Ponerini, the monophyly of *Pachycondyla* and of certain *Pachycondyla* subgenera, the phylogenetic position of *Harpegnathos*, and the rooting of Ponerini.

Divergence dating. The time scale of ponerine evolution was estimated by analyzing the ALL_DATA matrix in BEAST. The methods were identical to those used for the basic phylogenetic inferences in BEAST, except that the relaxed molecular clock was calibrated by inclusion of 23 non-redundant minimum age constraints on internal nodes (Supp. Table S3). These constraints are derived from the fossil record of ants and other aculeate hymenopterans and generally represent a subset of those employed by Brady *et al.* (2006). My denser sampling within Ponerinae enabled inclusion of additional ponerine fossils relative to that study, though my more limited

sampling outside Ponerinae precluded the use of several of their constraints. In a few cases I used more conservative fossil age estimates than used by Brady *et al.*, and I excluded a few fossils due to uncertainty in their phylogenetic positions. Fossil constraints were applied to the stem group of the clade to which they were thought to belong, usually based on the presence of morphological synapomorphies, though the proper assignment of *Pachycondyla succinea* (Mayr) was complicated by uncertainty in the phylogenetic relationships among *Pachycondyla* (*Pseudoponera*) and *Cryptopone* (see Supp. Table S3). Constraints were implemented in BEAST as uniform priors on node ages, with an upper bound equal to the assumed root age (see below) and a lower bound equal to the estimated fossil age.

Pristocera was constrained to be sister to the rest of the included taxa, such that the root node represented the most recent common ancestor (MRCA) of crown aculeates. The actual age of this node is unknown, so multiple dating analyses were run with a range of values for the fixed root age. The maximum and minimum plausible ages for this node were considered to be 185 and 145 Mya (million years ago), respectively, following the arguments of Brady *et al.* (2006): an upper bound of 185 Mya is almost certainly an overestimate due to its implied lack of fossilization of crown aculeates for a period of 45 Myr (million years). The lower bound of 145 Mya is reasonable given that crown aculeates appear in the fossil record about 140 Mya. The 185 and 145 Mya root ages are useful for providing upper and lower bounds for plausible internal node ages. Dating analyses were also performed using an assumed root age of 155 Mya, roughly the age for the MRCA of Aculeata estimated by Grimaldi and Engel (2005). I consider this latter root age to be the best estimate given current fossil evidence. Finally, I explored the effect of the aberrant CAD sequences for *Harpegnathos* on the date estimates by analyzing the data: (1) with all of CAD included for *Harpegnathos*; (2) with CAD 1nt/2nt excluded for *Harpegnathos*; and (3) with all of CAD excluded for *Harpegnathos*. MCMC burnin and total chain lengths for all dating analyses are given in Table 5.

Results and discussion

Phylogeny of Formicidae and position of Ponerinae

Bayesian analysis of the combined data set (ALL_DATA) yielded a phylogeny that is broadly congruent with the results of Moreau *et al.* (2006) and Brady *et al.* (2006) for subfamily-level relationships within Formicidae (Fig. 2), with strong support for a sister relationship between Leptanillinae and monophyletic poneroid and formicoid groups. The phylogeny differs from these earlier studies primarily in its inclusion of Martialinae, which is inferred as sister to the rest of Formicidae as in Rabeling *et al.* (2008).

A thorough investigation of the sister group of Ponerinae is outside the scope of this study, but Ponerinae was inferred to be sister to a clade consisting of *Paraponera* (Paraponerinae) + *Tatuidris* (Agroecomyrmecinae), with strong support in the MrBayes analysis (BPP = 1.00 for entire clade, BPP = 0.99 for *Paraponera* + *Tatuidris*) but much lower support in the RAxML bootstrap analysis (BS = 0.42 for the entire clade, BS = 0.43 for *Paraponera* + *Tatuidris*). This relationship was inferred by Moreau *et al.* (2006), and also by Brady *et al.* (2006) when their analyses included non-ant outgroups. In contrast, the analysis of Rabeling *et al.* (2008) reconstructed Ponerinae as sister to the formicoid subfamilies, as did the Formicidae-only analysis of Brady *et al.* (2006) under certain rootings. Given that these latter studies were unable to reject several alternate rootings of the phylogeny of Formicidae, the sister group of Ponerinae remains unresolved pending additional data. Future sampling of additional non-ant vespoid outgroups and leptanillines, and inclusion of additional genes, may provide better resolution of the basal relationships in Formicidae, including a firmer elucidation of the sister group of Ponerinae.

Monophyly of Ponerinae and its tribes and genera

The monophyly of Ponerinae was strongly supported by analyses of the ALL_DATA matrix (BPP = 1.00, BS = 0.98; Fig. 3), confirming the results of previous molecular phylogenetic studies with more limited sampling of ponerine taxa (*e.g.*, Moreau *et al.*, 2006; Brady *et al.*, 2006) and confirming predictions from morphological evidence. In his morphological synopsis of Ponerinae, Bolton (2003) noted two synapomorphies for the subfamily: fusion of the toruli to the frontal lobes, and the shape of the outer borders of the frontal lobes, which form ”simple

short semicircles or blunt triangles and in full-face view have a distinctly pinched-in appearance posteriorly". An examination of the inferred phylogeny of Formicidae and the subfamily-level morphological synopses of Bolton (2003) suggest an additional possible synapomorphy for Ponerinae, the loss of tergosternal fusion of the petiole (C. Schmidt and S. Shattuck, in prep), though this trait could conceivably be symplesiomorphic, with repeated evolution of a fused petiole in other poneroid lineages. All current ponerine genera not sampled in this study (*Asphinctopone*, *Belonopelta*, *Boloponera*, *Dolioponera*, *Feroponera*, and *Promyopias*) display these traits and their placements within Ponerinae and Ponerini are not in doubt, so it is highly unlikely that their inclusion in this study would have rendered Ponerinae non-monophyletic.

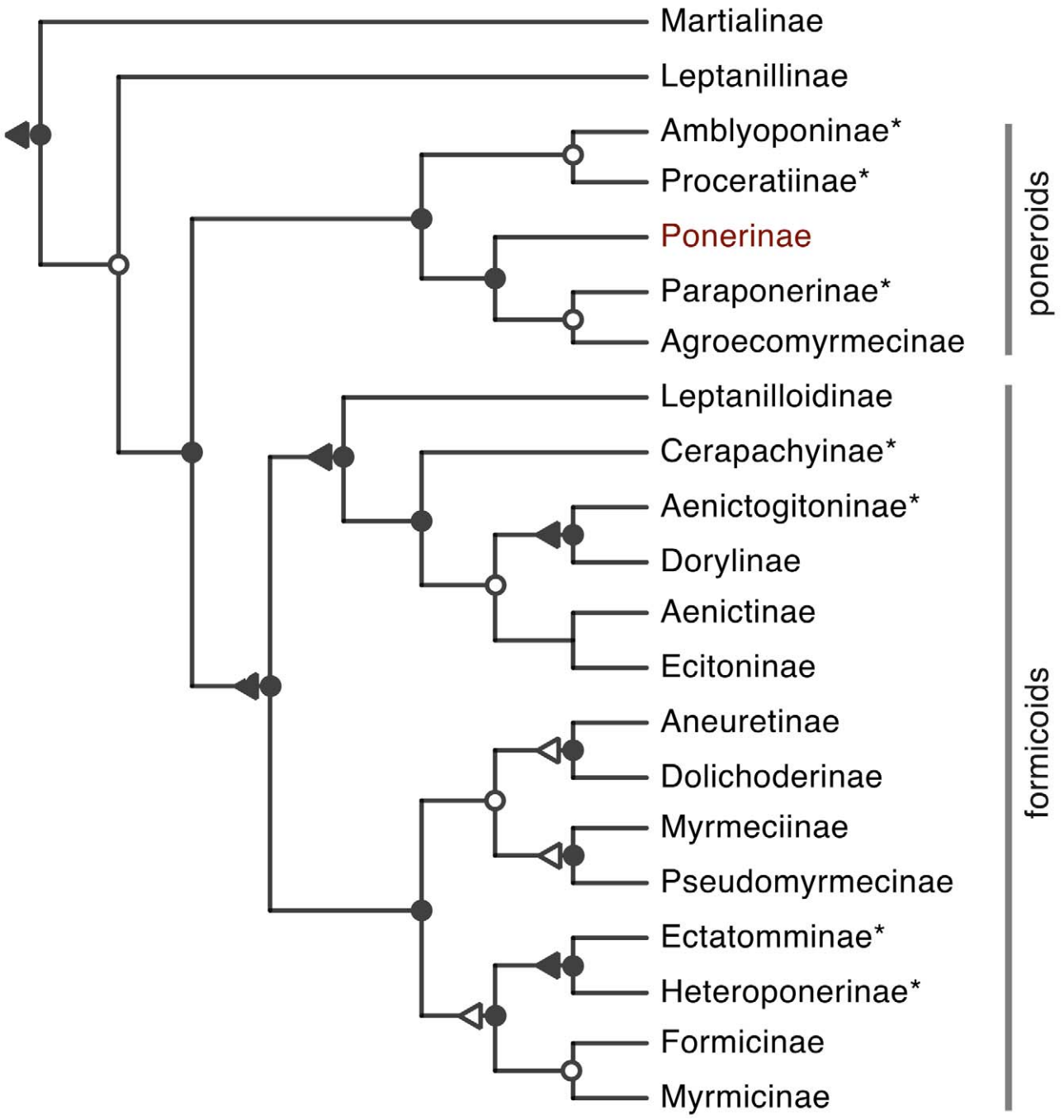


FIGURE 2. Consensus cladogram inferred by MrBayes analysis of ALL_DATA data set, showing relationships among ant subfamilies (non-ant outgroups not shown; the full tree is given in Supp. Fig. S1). Subfamilies that have at one time been included in Ponerinae are marked with an asterisk. Clade support is indicated by solid (MrBayes BPP = 1.00) and empty (0.95 < BPP < 0.99) circles and solid (RAxML BS = 1.00) and empty (0.95 < BS < 0.99) triangles. Cerapachyinae is probably actually non-monophyletic (Moreau *et al.*, 2006; Brady *et al.*, 2006).

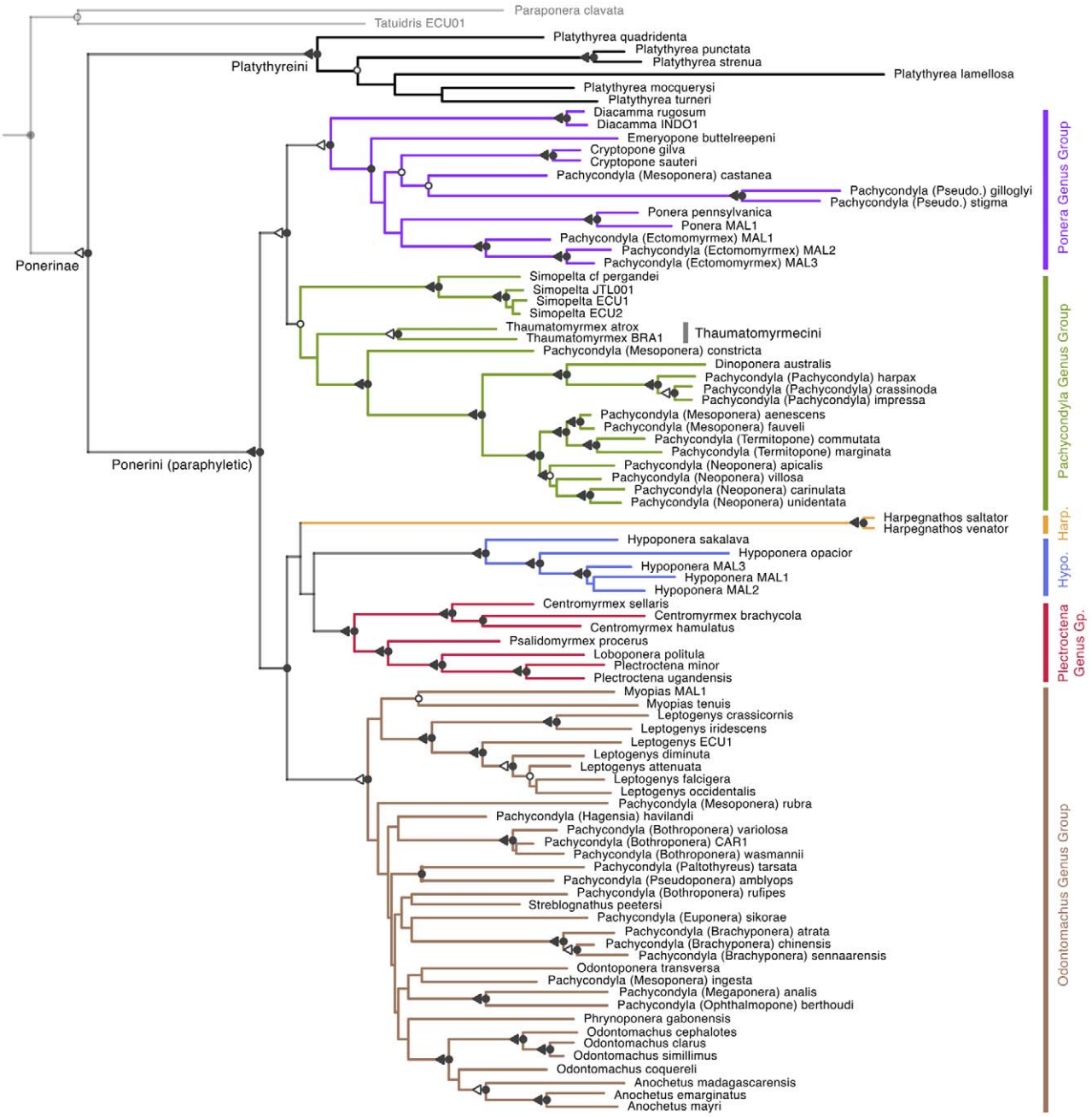


FIGURE 3. Consensus phylogeny of Ponerinae inferred by MrBayes analysis of ALL_DATA matrix with CAD included for *Harpegnathos*, showing mean branch lengths. Most outgroups are not shown (the full tree including all outgroups is given in Supp. Fig. S1). Genus groups within Ponerini are demarcated by branch color and by the labels on the right. Branches are colored for the crown groups of all genus groups except *Harpegnathos*, whose stem branch is also colored to ease visualization. Clade support is indicated by solid (MrBayes BPP = 1.00) and empty (0.95 < BPP < 0.99) circles and solid (RAxML BS = 1.00) and empty (0.95 < BS < 0.99) triangles. Pseudo.: *Pseudoponera*.

Phylogenetic analysis of the ALL_DATA matrix (Fig. 3) strongly supported the monophyly of Platythyreini (*Platythyrea*) (BPP = 1.00, BS = 1.00). The status of the genus *Eubothroponera* as a junior synonym of *Platythyrea* (Brown, 1952, 1975) is here confirmed with molecular data for the first time: *P. turneri*, though never formally included in *Eubothroponera* despite its obvious close relationship to members of that former genus, is nested within *Platythyrea* with strong support (monophyly of *Platythyrea* excluding *P. turneri*: BPP < 0.0001; BS = 0.002). Members of *Eubothroponera* have several morphological traits that differ from most other *Platythyrea* species, including coarser sculpturing, denser standing pilosity but sparser pubescence, and a relatively low helcium. The latter character approximates the condition found in most Ponerini, but the results of this study imply that low helcia were independently derived in *Platythyrea* and Ponerini.

The monophyly of the diverse tribe Ponerini was not supported in either Bayesian or ML bootstrap analyses (BPP < 0.0001; BS < 0.001), though Ponerini monophyly could not be rejected by an SH test (*p*-value = 0.661). The non-monophyly of Ponerini stems from its inclusion of Thaumatomyrmecini (*Thaumatomyrmex*), a result also recovered by Brady *et al.* (2006). The phylogenetic position of *Thaumatomyrmex* has been unclear due to its bizarre structure (including pitchfork-like mandibles, widely separated frontal lobes, and unusually convex eyes; Kempf, 1975). Bolton (2003) noted that *Thaumatomyrmex* shares several apomorphies with Ponerini, including vestigial male mandibles and articulation of the petiole low on the anterior face of the first gastral segment, but maintained its separate tribal status. The genus lacks a principal synapomorphy of Ponerini (the narrow insertion of the clypeus between the frontal lobes, and related characters; Bolton, 2003), but this character is highly modified in *Thaumatomyrmex*, rendering it uninformative about the phylogenetic position of the genus. Morphological data therefore imply at least a sister relationship between *Thaumatomyrmex* and Ponerini, and are consistent with its placement within Ponerini. The molecular results reported here suggest that *Thaumatomyrmex* is simply a highly derived member of Ponerini, its strange morphological traits secondarily derived as a result of extreme prey specialization (Brandão, 1991).

Among sequenced taxa, all sampled ponerine genera were strongly inferred to be monophyletic (BPP and BS = 1.00 for all genera except *Myopias*, for which BPP and BS both = 0.99, and *Thaumatomyrmex*, for which BS = 0.99) except *Pachycondyla* and *Odontomachus*. *Pachycondyla* was strongly inferred to be non-monophyletic (BPP of monophyletic *Pachycondyla* < 0.0001; BS < 0.001; SH *p*-value < 0.00001), as members of this genus were broadly distributed across the phylogeny of Ponerini. The sampled *Pachycondyla* species represent numerous distinct lineages which largely correspond to the boundaries of *Pachycondyla* "subgenera," though *Bothroponera*, *Mesoponera* and *Pseudoponera* are themselves non-monophyletic (*Bothroponera*: BPP < 0.001, BS = 0.014, SH *p*-value = 0.768; *Mesoponera*: BPP < 0.0001, BS < 0.001, SH *p*-value < 0.00001; *Pseudoponera*: BPP < 0.0001, BS < 0.001, SH *p*-value < 0.001).

Pachycondyla is the end product of heavy synonymization at the genus level, due primarily to the work of W. L. Brown, who was in the process of revising ponerine taxonomy at the time of his death. Brown's formal revision remains unpublished, but his numerous planned synonymizations have been published elsewhere without supporting justification (Brown, 1973; Snelling, 1981; Hölldobler and Wilson, 1990; Bolton, 1994; see also Mackay and Mackay, 2010) and in practice have been generally accepted by the scientific community. The monophyly of *Pachycondyla* is doubtful even in the absence of molecular data because the genus displays substantial morphological, ecological and behavioral diversity (reviewed in C. Schmidt and S. Shattuck, in prep.), is defined only by morphological symplesiomorphies such as triangular mandibles and paired metatibial spurs, and contains several lineages that show clear morphological similarities to other ponerine genera. This study confirms the extensive non-monophyly of *Pachycondyla* and stresses the need for a comprehensive taxonomic revision of ponerine genera (provided in C. Schmidt and S. Shattuck, in prep.).

Analysis of the ALL_DATA matrix was ambiguous about *Odontomachus* monophyly, with three resolutions: *O. coquereli* Roger sister to *Anochetus* (BPP = 0.58, BS = 0.37), *Odontomachus* monophyletic (BPP = 0.29, BS = 0.41), or *O. coquereli* sister to *Anochetus* + other *Odontomachus* (BPP = 0.13, BS = 0.22). To further address this question, a preliminary expanded data set was produced which included sequences for six additional *Anochetus* taxa and 18 additional *Odontomachus* taxa, plus data for the mitochondrial gene cytochrome oxidase I (COI), but excluding abdA, EF1αF1, and EF1αF2 (C. Schmidt, unpublished data). Phylogenetic analyses of these data supported reciprocal monophyly of *Odontomachus* and *Anochetus* (results not shown), but some phylogenetically important *Anochetus* species (such as members of the *A. gladiator* (Mayr) and *A. cato* Forel species groups; Brown, 1976, 1978) were not sampled and their inclusion in future phylogenetic analyses could still reveal the non-monophyly of either genus.

Genus groups within Ponerini

The ALL_DATA phylogeny resolves four major multi-generic clades within Ponerini (Fig. 3) which were previously unsuspected and are here given informal genus group names after the oldest included genus name (with abbreviations used in subsequent tables and figures): the *Ponera* ("PON"), *Pachycondyla* ("PACHY"), *Plectroctena* ("PLECTRO"), and *Odontomachus* ("ODONTO") genus groups, plus the single genera

Harpegnathos ("HARP") and *Hypoponera* ("HYPO"). In the ALL_DATA analysis these genus groups are all individually supported with MrBayes BPP of 1.00 except the *Pachycondyla* group (BPP = 0.99). Maximum likelihood (RAxML) BS support is generally lower for these clades, with only HARP, HYPO and PLECTRO supported with BS of 1.00, while ODONTO and PON have BS of 0.99. PACHY has a much lower BS of 0.64 due to uncertainty in the positions of *Simopelta* and *Thaumatomyrmex*, which are variously placed individually or in combination as sister to PON, though with low support.

The relationships among these genus groups are poorly resolved in the ALL_DATA phylogeny. The MrBayes phylogeny inferred from the ALL_DATA matrix infers the basal split in Ponerini as being between PON + PACHY (BPP = 1.00) and the remainder of Ponerini (BPP = 1.00), but RAxML gives lower support to this basal split (PON + PACHY: BS = 0.96; all other groups together: BS = 0.79). Relationships among HARP, HYPO, PLECTRO, and ODONTO are unresolved in both the MrBayes and RAxML analyses, with HYPO and PLECTRO most frequently inferred as sisters with very low support (BPP = 0.52, BS = 0.41).

The poor resolution among these genus groups is largely due to uncertainty in the position of *Harpegnathos*, which masks otherwise well-supported relationships. When *Harpegnathos* is trimmed from the topology of the sampled trees (post-inference), BS support increases marginally for the basal split between PON + PACHY (BS = 0.97) and HYPO + PLECTRO + ODONTO (BS = 0.81), but support for a HYPO + PLECTRO clade increases dramatically (BPP = 0.99, BS = 0.77). A MrBayes analysis of the ALL_DATA matrix with *Harpegnathos* removed prior to tree inference recovered identical support values for these relationships, indicating that the inclusion of *Harpegnathos* does not significantly affect the inference of relationships among other ponerine taxa and that uncertainty in the position of *Harpegnathos* is obscuring a well-supported backbone phylogeny of ((PON, PACHY), ((HYPO, PLECTRO), ODONTO)).

To further assess the level of support for these genus groups and their relationships, determine the degree of congruence among sampled genes, and evaluate the effect of missing data, I analyzed restricted subsets of the data in both MrBayes and RAxML. The principal concerns were that CAD, which makes up a substantial fraction of the data matrix, could be masking alternate topologies that are better supported by the other genes, and that the lack of CAD sequences for most outgroups, and the lack of secondary gene sequences for most ponerines, could be causing unreliable phylogenetic reconstructions within Ponerini. I was here interested only in estimating the degree of support for genus groups and more inclusive clades, regardless of the position of *Harpegnathos* and the rooting of Ponerini (these latter issues are dealt with separately below). *Harpegnathos* and all non-Ponerini taxa were trimmed from the sampled trees prior to calculation of posterior probabilities and bootstrap proportions, and trees were treated as unrooted. The following data sets were analyzed: ALL_DATA, CORE_DATA, ALL_BUT_CAD, CAD, WG_LWR_28S, and SEC_GENES. The relevant results from these analyses are summarized in Table 6. The full phylogenies from these analyses are given in Supp. Figs. S1–S5.

TABLE 6. Summary of support for major bipartitions within Ponerini across data subsets and analytical methods. *Harpegnathos* and all non-Ponerini taxa were trimmed from the sampled trees before calculation of posterior probabilities and bootstrap values, and trees were treated as unrooted. Results are given as MrBayes posterior probabilities / RAxML bootstrap proportions. Results for the ALL_DATA, CORE_DATA and CAD matrices are from the analyses with all of CAD included for *Harpegnathos*. See text section 3.3 for an explanation of genus group abbreviations. "*": Remainder of Ponerini. "n/a": Not applicable (relevant taxa not present in data set).

Bipartition	ALL_DATA	CORE_DATA	ALL_BUT_CAD	CAD	WG_LWR_28S	SEC_GENES[2]
PON/*	1.00 /0.99	1.00 /1.00	0.72 /0.54	1.00 /0.97	0.50 /0.48	n/a
PACHY/*	0.99 /0.65	0.99 /0.73	0.38 /0.32	0.84 /0.57	0.07 / 0.29	0.98 /0.96
PLECTRO/*	1.00 /1.00	1.00 /1.00	1.00 /0.98	1.00 /0.99	1.00 / 0.90	1.00 /0.95
ODONTO/*	1.00 /1.00	1.00 /1.00	1.00 /0.97	1.00 /1.00	1.00 /0.97	1.00 /1.00
PON+PACHY/*	1.00 /1.00	1.00 /1.00	0.79 /0.55	1.00 /0.99	0.97 /0.53	n/a
HYPO+PLECTRO/*	0.99 /0.78	0.72 /0.57	0.80 /0.48	0.94 / 0.60	0.50 / 0.38	0.94 /0.68

1 The SEC_GENES matrix has a much smaller sampling of ponerine taxa relative to the other matrices, so its clades are not necessarily directly comparable to those in the other matrices (this is especially relevant for PACHY).

The ALL_DATA and CORE_DATA data sets broadly agreed in their inferred support for the genus groups and for their inter-relationships, with the most significant difference being in their support for HYPO + PLECTRO (much lower support in the CORE_DATA analysis). This suggests that the inclusion of outgroups or (more likely) the inclusion of the secondary genes provides strong additional support for this bipartition. Both data sets give only modest support for the monophyly of PACHY in the RAxML bootstrap analysis but support this bipartition with a BPP of 0.99.

The ALL_BUT_CAD phylogeny differed from the ALL_DATA phylogeny in providing generally lower support for major clades, but does not strongly support any alternative relationships. ALL_BUT_CAD only weakly supports the respective monophyly of PACHY and PON, only weakly supports a clade consisting of PACHY + PON and gives similarly low support to HYPO + PLECTRO. The phylogenies inferred from the CAD, WG_LWR_28S, and SEC_GENES matrices are broadly congruent on most aspects of the phylogeny of Ponerini. The WG_LWR_28S phylogeny does not support reciprocal monophyly of PON and PACHY (the positions of *Simopelta* and *Thaumatomyrmex* are ambiguous) and gives very weak support to HYPO + PLECTRO, but does not strongly support any alternative relationships. CAD suggests a phylogeny for Ponerini that is broadly congruent with the other genes (ALL_BUT_CAD) but with generally higher support.

In sum, there is little indication that the significant gaps in the data matrix (CAD for most outgroups and secondary genes for most ponerines) are adversely affecting the inference of relationships within Ponerini. CAD alone resolves the major bipartitions within Ponerini (those present in the ALL_DATA analysis) with generally high support, but the other core genes (wg, LW Rh and 28S) together are ambiguous about relationships at this level. The secondary genes together resolve the same bipartitions as CAD with high support, but the relatively small number of ponerine taxa sampled for these genes (particularly the absence of any members of PON) urges caution in interpreting the bipartition support from these genes. Additional taxon sampling within Ponerini for these secondary genes and newly-developed genes such as *arginine kinase* and *enolase* (C. Schmidt, unpublished data; Ward *et al.*, 2010) should be a priority in any future molecular phylogenetic studies of Ponerinae. Unfortunately, a preliminary consideration of morphological characters has generally not revealed any clear apomorphies useful for determining relationships among genus groups within Ponerini (C. Schmidt and S. Shattuck, in prep.), though morphology does support certain individual genus groups (Section 3.5) and may inform the phylogenetic position of *Harpegnathos* (Section 3.4).

Phylogenetic position of *Harpegnathos* and rooting of Ponerini

The phylogenetic position of *Harpegnathos* is poorly resolved in the analysis of the ALL_DATA matrix. Some of this uncertainty may stem from the CAD sequences of *Harpegnathos*, which have a large number of unusual amino acid changes relative to other ponerines and thus place *Harpegnathos* on a long branch (Fig. 3). To evaluate the phylogenetic position of *Harpegnathos*, I examined the *Harpegnathos* CAD sequences in greater detail by performing single gene analyses of: (1) all of CAD; (2) only first and second positions (1nt/2nt) of CAD; and (3) only third positions (3nt) of CAD. The MrBayes analysis of the complete CAD data set inferred a remarkably long branch for *Harpegnathos* (Fig. 4A), grouped it with HYPO, PLECTRO, and ODONTO with a BPP of 0.95, and placed it most frequently as sister to HYPO (BPP = 0.72). The MrBayes analysis of CAD 1nt/2nt (Fig. 4B) inferred an even longer branch for *Harpegnathos*, and again placed it among the aforementioned genus groups (BPP = 0.93), though its position within this clade was highly ambiguous. The MrBayes analysis of CAD 3nt (Fig. 4C) recovered a much more typical branch length (relative to other taxa) for *Harpegnathos* and had generally low support for relationships among the genus groups, though it placed *Harpegnathos* as sister to the rest of Ponerini (BPP = 0.57).

The characteristics of the *Harpegnathos* CAD sequences suggest that their high rate of amino acid change may be the result of positive selection. The sequences align easily with other ponerine CAD sequences (except in a hypervariable region toward the 5' end of the first sequenced fragment), contain introns at the same locations as in other ants, and are devoid of stop codons, suggesting that they are not functionless pseudogenes (Balakirev and Ayala, 2003). The *Harpegnathos* CAD sequences were obtained using three independent sets of nested primers (all successfullly used with other ponerines), but the overlapping regions of the resulting sequence fragments agreed perfectly and I observed no hint of secondary amplicons which would suggest multiple gene copies. In addition, the

inferred position of *Harpegnathos* in the single gene analyses of CAD (*i.e.*, nested within Ponerini: Fig. 4A-B) suggests that these sequences are not phylogenetically deep paralogs (Kuzniar *et al.*, 2008), and CAD paralogs have not been reported in any holometabolous insects to date (Moulton and Wiegmann, 2004; Danforth *et al.*, 2006; Wild and Maddison, 2008; Ward *et al.*, 2010). Most directly, a BLAST search of the recently-published genome of *Harpegnathos saltator* Jerdon (Bonasio *et al.*, 2010) revealed a single match for the *H. saltator* CAD sequence obtained in the present study (accession number HSAL15216-RA, with 100% identity for the entire 1,926 bp sequence; sequence accessed at the Hymenoptera Genome Database, http://hymenopteragenome.org/ant_genomes/; Munoz-Torres *et al.*, 2011). No other match was found within the *H. saltator* genome, indicating the absence of paralogs or pseudogenes.

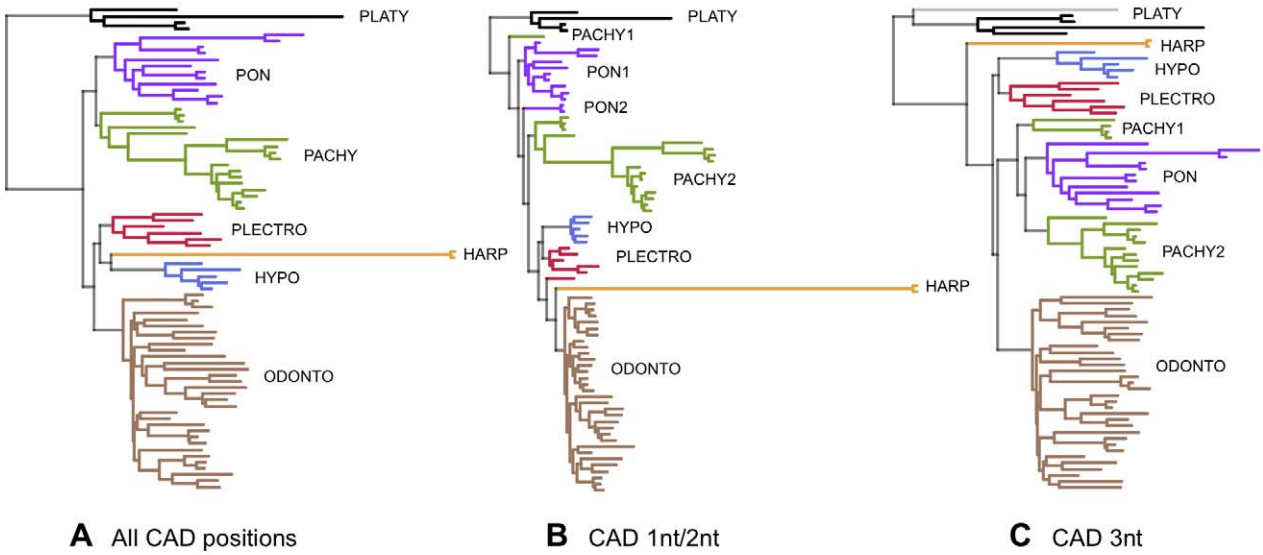


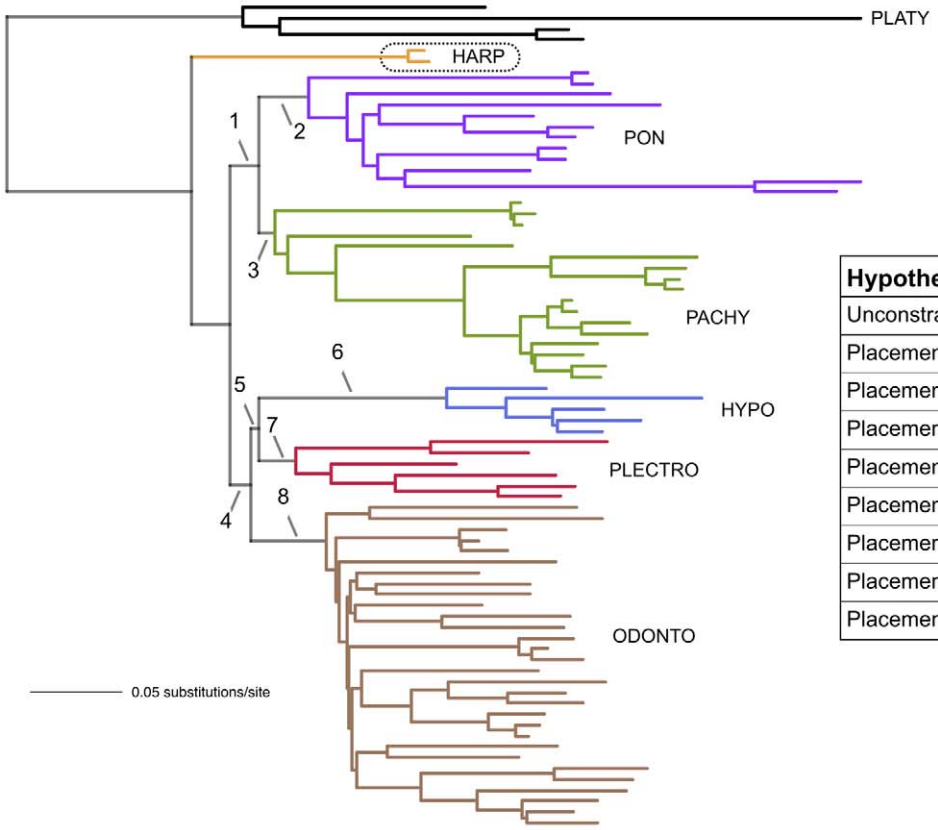
FIGURE 4. Results of single gene MrBayes analyses of (A) all of CAD, (B) first and second codon positions of CAD, and (C) third codon positions of CAD. Only ponerine taxa are shown, except in C, in which *Mystrium* (Amblyoponinae, light gray branch) was reconstructed as sister to *Platythyrea* with low support. Branch colors for genus groups are as in Fig. 3. Clade support and taxon labels are not indicated except for genus group abbreviations (see text).

While the CAD sequences obtained for *Harpegnathos* do not appear to represent pseudogenes or paralogs, their high number of unusual non-synonymous substitutions relative to other ponerines may have resulted from selection. The unremarkable branch length of *Harpegnathos* in the analysis of CAD 3nt, in contrast to the long branch from CAD 1nt/2nt, suggests a high ratio of non-synonymous to synonymous changes characteristic of positive selection. If selection was truly responsible for shaping these atypically divergent sequences, they would severely violate the assumption of stationary evolutionary processes on which the utilized phylogenetic reconstruction methods are based (Felsenstein, 2003; Gadagkar and Kumar, 2005). The sequences cannot definitively be shown to violate these assumptions, but caution is necessary in utilizing them as phylogenetic markers. I therefore analyzed the ALL_DATA matrix with: (1) all of CAD included for *Harpegnathos*; (2) only third positions included (justified if the gene has been under strong selection, as third positions should still evolve relatively neutrally given their high redundancy); and (3) all of CAD excluded for *Harpegnathos*.

Analysis of the ALL_DATA matrix with CAD 1nt/2nt excluded for *Harpegnathos* resulted in *Harpegnathos* being reconstructed as sister to the rest of Ponerini with high Bayesian support (BPP: 1.00), but only moderate ML support (BS: 0.70). Relationships among the other genus groups were identical to the analysis of the full ALL_DATA matrix (see Fig. 3), with HYPO and PLECTRO inferred as sister groups with high support (BPP / BS: 0.99 / 0.93) and inferred as sister to ODONTO (BPP / BS: 1.00 / 0.64). Analysis of the ALL_DATA matrix with all of CAD excluded for *Harpegnathos* resulted in greater ambiguity regarding its position, with *Harpegnathos* inferred as sister to the rest of Ponerini (BPP / BS: 0.23 / 0.33) or to ODONTO (BPP / BS: 0.61 / 0.34).

Alternate phylogenetic positions of *Harpegnathos* were evaluated using the likelihood-based Shimodaira-Hasegawa test, which is conservative in its assessment of alternative hypotheses (Shimodaira, 2002; Aris-Brosou, 2003). For this analysis I utilized the CORE_DATA matrix with all of CAD excluded for *Harpegnathos*, since this

data set should be most conservative in evaluating alternative hypotheses of *Harpegnathos* placement. The unconstrained topology placed *Harpegnathos* as sister to the rest of Ponerini, but none of the tested alternative placements could be rejected (Fig. 5). The inferred placement of *Harpegnathos* as sister to the rest of Ponerini could result from long branch attraction (Felsenstein, 2003; Bergsten, 2005) to *Platythyrea* and the outgroups, given the long stem branches of *Platythyrea*, *Harpegnathos*, and Ponerini. I therefore compared MrBayes analyses of the ALL_DATA and PONERINI_ONLY data sets to evaluate the impact of outgroups on inferred relationships within Ponerini.



Hypothesis	-lnL	*p*-value
Unconstrained	40264.305	1.000
Placement 1	40267.712	0.616
Placement 2	40272.845	0.295
Placement 3	40272.410	0.321
Placement 4	40267.470	0.635
Placement 5	40267.612	0.620
Placement 6	40268.413	0.556
Placement 7	40268.437	0.555
Placement 8	40266.270	0.519

FIGURE 5. Phylogeny of Ponerinae inferred by RAxML analysis of the CORE_DATA matrix, with all of CAD excluded for *Harpegnathos*. Branch colors for genus groups are as in Fig. 3. Clade support and taxon labels are not shown except for genus group abbreviations. Numbers indicate alternative placements of *Harpegnathos* (bounded clade) evaluated with an SH test (inset table).

The analysis of PONERINI_ONLY with all of CAD included for *Harpegnathos* yielded a result similar to the corresponding ALL_DATA analysis (see Fig. 3): *Harpegnathos* formed a clade or bipartition with HYPO and PLECTRO, though with low support (BPP: 0.75 in ALL_DATA; BPP: 0.57 in PONERINI_ONLY). Analysis of PONERINI_ONLY with CAD 1nt/2nt excluded for *Harpegnathos* yielded a different inferred position for *Harpegnathos* than the corresponding ALL_DATA analysis (Fig. 6). The ALL_DATA analysis (Fig. 6A) placed *Harpegnathos* as sister to the rest of Ponerini with high support (BPP = 1.00), with HYPO and PLECTRO as sister to ODONTO (BPP = 1.00). In contrast, the unrooted PONERINI_ONLY analysis (Fig. 6B) placed *Harpegnathos* with HYPO and PLECTRO, but with low support (BPP: 0.74). The PONERINI_ONLY result is not inconsistent with the ALL_DATA result but is more ambiguous and suggests that *Harpegnathos* is being attracted to the outgroups (including *Platythyrea*), though whether this is artifactual long branch attraction is unclear. Analyses of both the ALL_DATA and PONERINI_ONLY matrices with all of CAD excluded for *Harpegnathos* inferred *Harpegnathos* as forming a clade or bipartition with ODONTO, though with relatively low support (BPP: 0.61 in ALL_DATA; BPP: 0.81 in PONERINI_ONLY).

These results indicate support for three possible placements of *Harpegnathos*: (1) sister to the rest of Ponerini (supported by ML analysis of ALL_DATA with CAD excluded for *Harpegnathos* and by MrBayes analysis of ALL_DATA with CAD 1nt/2nt excluded for *Harpegnathos*); (2) forming a clade with HYPO and PLECTRO

(supported by MrBayes analysis of ALL_DATA); or (3) sister to ODONTO (supported by MrBayes analysis of ALL_DATA with CAD excluded for *Harpegnathos*).

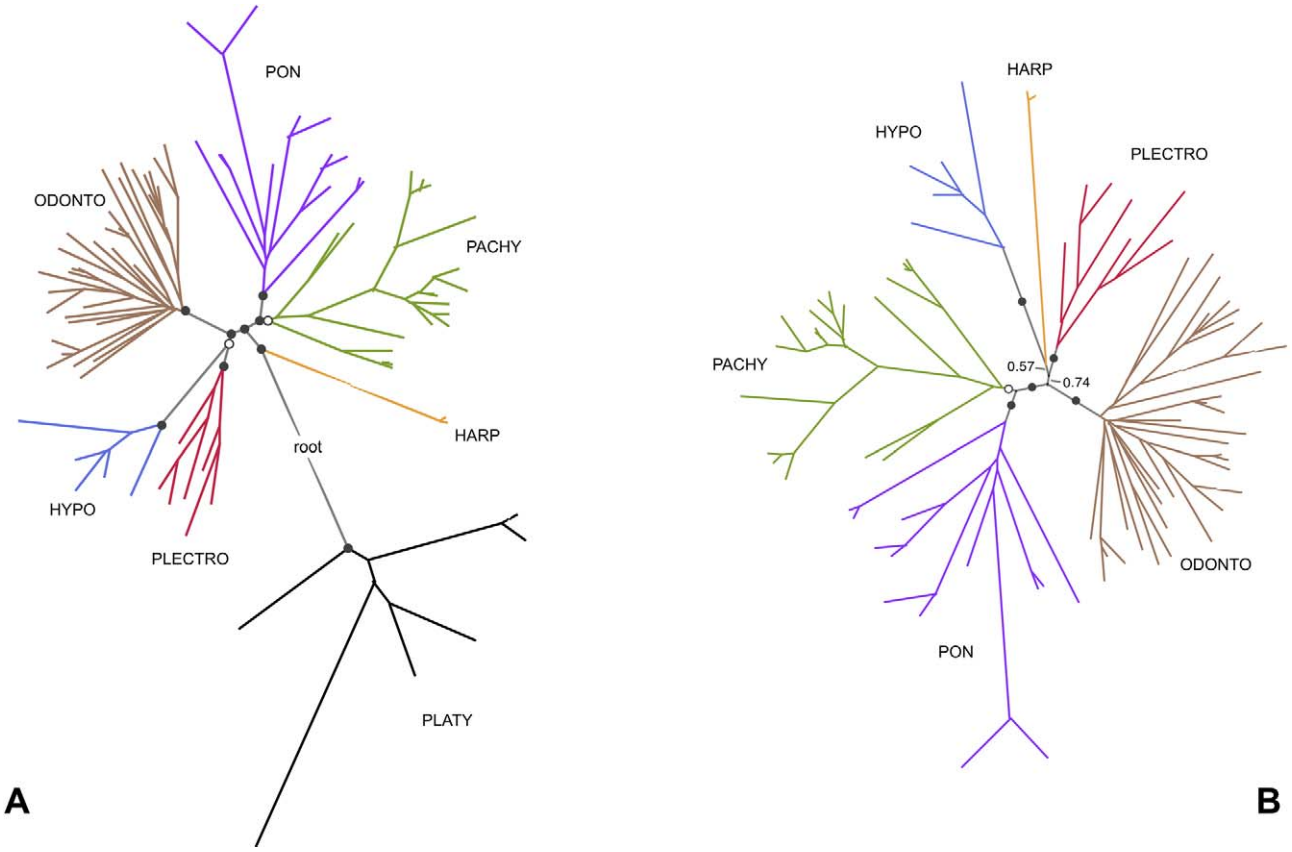


FIGURE 6. Comparison of the inferred placements of *Harpegnathos* (HARP) in MrBayes analyses of (A) the ALL_DATA matrix with CAD 1nt/2nt excluded for *Harpegnathos*, and (B) the PONERINI_ONLY matrix with CAD 1nt/2nt excluded for *Harpegnathos*. Outgroups are not shown in A, and the tree is drawn as unrooted despite an inferred root location (indicated). The phylogeny in B is unrooted. Branch colors for genus groups are as in Fig. 3. Clade or bipartition support is indicated by solid circles (MrBayes BPP = 1.00), empty circles (0.95 < BPP < 0.99), or numbers, and is shown only for select nodes or branches. The trees are not drawn to the same scale.

To further evaluate the position of *Harpegnathos* and to determine the influence of outgroup sequences on the inferred relationships within Ponerini, I quantified support for alternate rootings of the tribe (Table 7). This was done by calculating BPP for these rootings in MrBayes analyses of the ALL_DATA matrix and root-inferred BEAST analyses of the PONERINI_ONLY matrix, under the three scenarios for inclusion of CAD for *Harpegnathos*. I also tested several potential rootings of Ponerini using an SH test of the CORE_DATA matrix, with all of CAD excluded for *Harpegnathos*. The tested root locations corresponded with the tested placements of *Harpegnathos* given in Fig. 5, with the addition of a topology in which *Harpegnathos* was constrained to be sister to the rest of Ponerini and PON, PACHY and ODONTO were further constrained to form a clade. Such a topology is implied by a hypothetical rerooting of the ALL_DATA tree (Fig. 3) at *Harpegnathos*, by the CAD 3nt tree (Fig. 4C), and by a single gene analysis of wg (result not shown).

None of the tested rootings could be rejected by the SH test, likely reflecting the long stem branch and very short internal branches at the base of Ponerini. A rooting at *Harpegnathos* is supported by the ML analysis and by the MrBayes analysis of ALL_DATA with CAD 1nt/2nt excluded for *Harpegnathos*, while a rooting at PON + PACHY is supported by MrBayes analyses of ALL_DATA with all of CAD either included or excluded for *Harpegnathos*. The BEAST analyses were comparatively insensitive to the presence of CAD for *Harpegnathos*, as all three variants of the PONERINI_ONLY data set yielded similar support values for a rooting at PON + PACHY (BPP ranged from 0.46 to 0.50), with various support for other rootings.

In sum, molecular data best support a rooting of Ponerini either at *Harpegnathos* or at PON + PACHY. It is unlikely that long branch attraction is responsible for the inferred rooting at *Harpegnathos* in some analyses. Long

branch attraction between *Harpegnathos* and the outgroups would be most likely to occur with inclusion of *Harpegnathos* CAD 1nt/2nt data, since they are responsible for the longest stem branch for *Harpegnathos*. This is not what is observed, as *Harpegnathos* is drawn toward the outgroups only when its CAD 1nt/2nt data, and their possibly artifactual signal, are excluded. A sister relationship between *Harpegnathos* and the rest of Ponerini is also inferred by analysis of the WG_LWR_28S matrix, though not with strong support (Supp. Fig. S4).

TABLE 7. Evaluation of alternative rootings of Ponerini. Rootings indicate one side of the basal split. Genus group abbreviations are given in the text. Likelihoods and p-values are from an SH test of the CORE_DATA matrix with all of CAD excluded for *Harpegnathos*. Bayesian posterior probabilities (BPP) are given for MrBayes analyses of the ALL_DATA matrix and rooted BEAST analyses of the PONERINI_ONLY matrix, with: (1) all of CAD included for *Harpegnathos*; (2) CAD 1nt/2nt excluded for Harpagnathos; or (3) all of CAD excluded for *Harpegnathos*. Not all potential rootings were evaluated. "n/a": Not applicable.

	SH Test		MrBayes BPP			BEAST BPP		
Rooting	-lnL	p-value	1	2	3	1	2	3
HARP[1]	40264.305	1.000	0.0041	0.9991	0.2341	0.1191	0.1001	0.0171
w/ monophyletic (PON+PACHY+ODONTO)	40270.440	0.613	< 0.0001	< 0.0001	< 0.0001	0.034	0.037	< 0.001
PON	40274.762	0.451	< 0.0001	< 0.0001	< 0.0001	0.126	0.083	0.234
PACHY	40274.762	0.451	< 0.0001	< 0.0001	< 0.0001	0.090	0.062	0.082
HYPO	40272.241	0.571	< 0.0001	< 0.0001	< 0.0001	0.042	0.051	0.022
PLECTRO	40273.787	0.473	< 0.0001	< 0.0001	< 0.0001	0.011	0.008	0.030
ODONTO	40268.288	0.658	< 0.001	< 0.0001	0.001	0.047	0.062	0.010
PON+PACHY	40266.205	0.874	0.995	< 0.001	0.756	0.455	0.495	0.462
HYPO+PLECTRO+ODONTO	40267.712	0.775	< 0.0001	< 0.0001	0.006	< 0.001	0.001	0.001
HYPO+PLECTRO	40273.242	0.497	< 0.0001	< 0.0001	< 0.001	0.019	< 0.001	0.037
All other rootings combined	n/a	n/a	< 0.0001	< 0.0001	0.003	0.079	0.138	0.105

1 Inclusive of the rooting below ("HARP w/ monophyletic (PON+PACHY+ODONTO)").

Morphological evidence is consistent with a sister relationship between *Harpegnathos* and the remainder of Ponerini, as the genus shares several potentially plesiomorphic morphological character states with *Platythyrea* and *Paraponera* (one member of the putative sister group of Ponerinae). These characteristics, which are variously absent in most other Ponerini, include large size, large eyes, an obsolete metanotal groove, a broad propodeal dorsum, slit-shaped propodeal spiracles, laterally-opening metapleural gland orifices, toothed tarsal claws, prominent arolia, an elongated petiole, and articulation of the petiole near midheight on the anterior face of the first gaster segment. While these characters are all apparently homoplasious within Ponerini and could be convergent in *Harpegnathos*, *Platythyrea* and *Paraponera*, a sister relationship between *Harpegnathos* and the rest of Ponerini would imply the most parsimonious scenario of morphological evolution in Ponerini. (*Tatuidris*, the other member of the putative sister group of Ponerinae, is so derived that its morphological character states are difficult to interpret for this purpose.)

Relationships within genus groups

While relationships among the genus groups of Ponerini are somewhat unresolved, the genus-level relationships within those groups are generally well supported. Many of these relationships are also supported by morphological evidence (C. Schmidt and S. Shattuck, in prep.).

The *Ponera* group (PON) is strongly supported by molecular evidence, but clear morphological synapomorphies are lacking for the group. The basal split in the group is itself well-supported in the MrBayes analysis of ALL_DATA, with *Diacamma* inferred as sister to the rest of the group (BPP / BS: 1.00 / 0.87). Relationships among the remaining members of the group are poorly resolved. *Emeryopone* is reconstructed with

moderate support as sister to the remaining taxa (BPP / BS: 0.88 / 0.62), *Cryptopone* is inferred with fairly strong Bayesian support (BPP / BS: 0.98 / 0.45) to form a clade with *Pachycondyla* (*Pseudoponera s.s.*) + *Pachycondyla* (*Mesoponera*) *castanea* (Mayr) (for this latter clade, BPP / BS: 0.99 / 0.59), and *Ponera* and *Pachycondyla* (*Ectomomyrmex*) are inferred as sister groups with moderate support (BPP / BS: 0.91 / 0.67). These relationships are consistent with morphological evidence.

The *Pachycondyla* group (PACHY) is strongly supported by the MrBayes analysis of ALL_DATA (BPP: 0.99) but not by the RAxML bootstrap analysis (BS: 0.64). The group is characterized by a potential morphological synapomorphy newly recognized here: a U-shaped cuticular lip at the posterior margin of the metapleural gland orifice. All examined members of PACHY have this cuticular lip, though a similar structure is also present in *Diacamma* and may represent a synapomorphy of PACHY and PON (subsequently lost in most members of the latter group). Relationships within PACHY are well supported except at the base, where the relationships among *Simopelta*, *Thaumatomyrmex* and the rest of the group are unresolved. One side of the basal split is variously reconstructed as *Simopelta* (BPP / BS: 0.74 / 0.30), *Simopelta* + *Thaumatomyrmex* (BPP / BS: 0.18 / 0.18), or *Thaumatomyrmex* alone (BPP / BS: 0.07 / 0.17). The remaining lineages form a strongly resolved clade (BPP / BS: 1.00 / 1.00 for the entire clade and most internal nodes), with *Pachycondyla* (*Mesoponera*) *constricta* (Mayr) sister to a clade consisting of *Dinoponera* + *Pachycondyla* (*s.s.*) and a clade composed of *Pachycondyla* (*Neoponera*), *Pachycondyla* (*Termitopone*), and some *Pachycondyla* (*Mesoponera*) species. These relationships are consistent with morphological evidence, though morphological data are ambiguous about the positions of *Simopelta* and *Thaumatomyrmex*.

The *Odontomachus* group (ODONTO) is unequivocally supported by molecular evidence and is subtended by a long stem branch, but morphological synapomorphies for the group are not apparent. Relationships within the group are very poorly resolved, suggesting the possibility of a relatively rapid radiation that left little information about the basal branching order. The basalmost split in this group is most frequently resolved as being between *Myopias* + *Leptogenys* and the remaining taxa in the group, though this is poorly supported (BPP / BS: 0.73 / 0.32 for *Myopias* + *Leptogenys*; BPP / BS: 0.53 / 0.16 for the remainder of the group). Support for this split increases significantly with the deletion of *Pachycondyla* (*Mesoponera*) *rubra* from the sampled trees, as this taxon is variously inferred as sister to *Myopias*, *Leptogenys*, *Myopias* + *Leptogenys*, or to the remainder of the group. This uncertainty likely stems from the relatively high proportion of missing data for *P. rubra*. With *P. rubra* removed, the *Myopias* + *Leptogenys* clade has a somewhat higher BPP of 0.88 (BS: 0.42) and the remainder of ODONTO has a much improved BPP of 0.84 (BS: 0.25), though both values are still low. Among the remaining lineages, only a few generic relationships are well-supported: *Pachycondyla* (*Megaponera*) and *Pachycondyla* (*Ophthalmopone*) are inferred as sisters (BPP / BS: 1.00 / 1.00), and *Pachycondyla* (*Paltothyreus*) and *Pachycondyla* (*Pseudoponera*) *amblyops* (Emery) are resolved as sisters (BPP / BS: 1.00 / 0.61). *Odontomachus* and *Anochetus* form a clade with a BPP and BS of 1.00, though their reciprocal monophyly is uncertain (see Section 3.2). All other genus-level relationships within the group are very poorly resolved.

A possible morphological synapomorphy for the *Plectroctena* group (PLECTRO) is their laterally-opening metapleural gland orifices, though this is also shared with *Harpegnathos* and *Platythyrea* and may be plesiomorphic. Relationships within PLECTRO are well resolved (all with BPP and BS of 1.00), with *Centromyrmex* sister to (*Psalidomyrmex* + (*Loboponera* + *Plectroctena*)). This topology is also strongly supported by morphological evidence.

Time scale of Ponerine evolution

The divergence dates estimated by this study for major clades within Formicidae are broadly similar to those of Brady *et al.* (2006) despite the use of different estimation methods (Bayesian relaxed clock and penalized likelihood, respectively; Sanderson, 2002; Drummond *et al.*, 2006) and a different root node (*Pristocera* was excluded from the dating analyses in Brady *et al.*). Table 8 summarizes the age estimates for major ant clades from this study, Brady *et al.* (2006), and Moreau *et al.* (2006). The entire dated phylogeny is given in Supp. Fig. S6. The estimates of Moreau *et al.* are substantially older than those from both this study and Brady *et al.* (2006). These discrepencies are difficult to evaluate because certain details of the methods in Moreau *et al.* (2006) were not given, but their results are inconsistent with the fossil record of Hymenoptera because they imply an implausibly long period without known fossilization of aculeates (Brady *et al.*, 2006).

TABLE 8. Comparison of age estimates for crown groups of select ant clades from this study, Brady *et al.* (2006) and Moreau *et al.* (2006). The latter two studies did not include Martialinae, so ages estimated in this study for crown Formicidae are given with and without that taxon included. Results for this study are from analyses with all of CAD included for *Harpegnathos*, for the three considered root ages. Results shown for Brady *et al.* (2006) are from their outgroup-rooted ("tree A") analyses, for the two considered root ages. Results for Moreau *et al.* (2006) are from their penalized likelihood analysis with minimum fossil ages used as constraints. Age estimates are given as "Mean (Upper 95% CI–Lower 95% CI)" and are rounded to the nearest MY. "n/a": Not applicable. "n/r": Not reported.

	This Study						Brady *et al.*				Moreau *et al.*	
Clade	185 Mya		155 Mya		145 Mya		185 Mya		145 Mya		n/r	
Formicidae (incl. Martialinae)	141	(152–129)	123	(130–116)	116	(122–111)	n/a		n/a		n/a	
Formicidae (excl. Martialinae)	133	(143–122)	118	(124–112)	111	(117–106)	133	(139–127)	116	(120–112)	141	(149–133)
Formicoids	114	(122–104)	104	(111–98)	100	(106–95)	119	(125–114)	105	(109-102)	125	(131–118)
Dorylomorphs	88	(101–75)	83	(94–72)	78	(95–66)	88	(94–82)	77	(82–72)	n/r	
Myrmeciomorphs	88	(99–76)	82	(91–74)	81	(89–72)	103	(109–97)	92	(97–87)	n/r	
Dolichoderomorphs	82	(97–62)	77	(92–62)	76	(89–62)	100	(106–94)	91	(95–87)	108	(113–103)
Formicinae	70	(82–58)	66	(76–56)	65	(75–56)	82	(86–78)	77	(81–74)	92	(92–92)
Myrmicinae	76	(88–65)	76	(85–66)	73	(82–65)	89	(95–83)	82	(86–78)	100	(104–96)
Poneroids	118	(128–107)	107	(115–99)	101	(108–93)	115	(123–107)	100	(106–94)	128	(134-122)
Amblyoponinae	100	(114–86)	89	(104–66)	85	(97–72)	106	(115–97)	92	(97–85)	113	(118–108)
Proceratiinae	88	(108–66)	75	(95–54)	74	(93–49)	90	(101–79)	78	(87–69)	111	(115–107)
Ponerinae	102	(112–92)	94	(104–85)	85	(96–78)	90	(98–82)	79	(85–73)	111	(117–104)
Ponerini	79	(89–71)	73	(82–67)	70	(77–60)	61	(67–55)	53	(58–48)	n/r	

I estimated the origin of crown Formicidae at 123 Mya (preferred age with a 155 Mya root age), with upper and lower bound estimates of 141 and 116 Mya, respectively. This upper bound estimate is older than that of Brady *et al.* but results from my inclusion of *Martialis*, which had not yet been described at the time of their study. My upper estimate for crown Formicidae excluding *Martialis* (133 Mya) is identical to theirs. Other areas of close agreement between these studies include the ages of the poneroid and formicoid groups, the dorylomorph subfamilies, and both Amblyoponinae and Proceratiinae.

The age estimates inferred in this study differ substantially from those of Brady *et al.* (2006) for several regions of the tree, however. I inferred significantly younger age estimates for the myrmeciomorph subfamilies, dolichoderomorph subfamilies, Formicinae, and Myrmicinae, and significantly older estimates for Ponerinae and Ponerini. Two non-exclusive factors may explain these differences. First, Brady *et al.* utilized the fossil taxon *Burmomyrma* as a minimum age constraint for the stem group of the dolichoderomorph subfamilies (and therefore also of their sister group, the myrmeciomorph subfamilies). *Burmomyrma* represents a powerful constraint given its significant age (100 Mya), yet its association with the dolichoderomorph subfamilies is tentative at best (Dlussky, 1996). I therefore felt that exclusion of this fossil was prudent, and my age estimates for the dolichoderomorph and myrmeciomorph subfamilies are consequently significantly younger than those of Brady *et al.* Using separate data and analyses from Brady *et al.*, but also using *Burmomyrma* as a minimum age constraint, Ward *et al.* (2010) estimated a crown-group age for the dolichoderomorphs that was comparable to that in the former study. The younger age estimates reported here for Formicinae and Myrmicinae are probably also partially influenced by the exclusion of *Burmomyrma*, but another factor may be the present study's lighter sampling of fossil constraints for these subfamilies. In practice, informative minimum age constraints increase the estimated ages of associated nodes (Near *et al.*, 2005; Benton and Donoghue, 2007; Hug and Roger, 2007; Rutschmann *et al.*, 2007), so the denser sampling of formicine and myrmicine fossils by Brady *et al.* likely increased their age estimates for these subfamilies. The same phenomenon may explain my older age estimates for Ponerinae and Ponerini.

The fossil-calibrated dated phylogeny of Ponerinae inferred from analysis of the ALL_DATA matrix in BEAST (with a 155 Mya root age and CAD included for *Harpegnathos*) is shown in Fig. 7. Divergence date

estimates within Ponerinae were relatively consistent across different root ages and with CAD sequences for *Harpegnathos* included or excluded (results summarized for select ponerine clades in Table 9), with the variability of age estimates generally increasing with node depth. The mean estimated age of stem Ponerinae (*i.e.*, the age of the MRCA of Ponerinae and its inferred sister group, *Paraponera* + *Tatuidris*) varied from 113 to 95 Mya depending on the assigned root age, with an estimated age of 103 Mya in the preferred analysis. This places the origin of stem Ponerinae around the transition between the Upper and Lower Cretaceous (99.6 Mya: International Commission on Stratigraphy, 2008). Estimates for the age of the basal split within Ponerinae ranged from 102 to 85 Mya, with a preferred age of 94 Mya, while the estimate for crown Ponerini ranged from 79 to 70 Mya, with a preferred age of 73 Mya.

TABLE 9. Age estimates for selected clades in Ponerinae, derived from analysis of the ALL_DATA matrix in BEAST. Ages are for crown groups unless otherwise noted. For analyses with a root age of either 185 or 145 Mya, results are shown only from fossil-calibrated analyses that included all of CAD for *Harpegnathos*. For analyses with a root age of 155 Mya, results are shown for fossil-calibrated analyses with (1) all of CAD included for *Harpegnathos*, (2) CAD 1nt/2nt excluded for *Harpegnathos* (the preferred analysis), and (3) all of CAD excluded for *Harpegnathos*. Age estimates are given as "Mean (Upper 95% CI–Lower 95% CI)" and are rounded to the nearest Myr.

	185 Mya Root		155 Mya Root						145 Mya Root	
Clade Analysis:	1		1		2		3		1	
Stem Ponerinae	113	(123–101)	103	(111–94)	101	(108–93)	100	(106–90)	95	(103–87)
Ponerinae	102	(112–92)	94	(104–85)	92	(100–83)	88	(96–79)	85	(96–78)
Platythyreini	62	(81–45)	62	(73–51)	57	(71–43)	53	(67–41)	58	(73–38)
Ponerini	79	(89–71)	73	(82–67)	76	(85–67)	67	(74–60)	70	(77–60)
Ponera / *Pachycondyla* groups	72	(82–64)	67	(76–59)	64	(72–58)	61	(68–55)	64	(73–56)
Hypoponera / *Plectroctena* groups	67	(78–56)	63	(73–54)	59	(69–52)	56	(64–48)	59	(67–51)
Ponera group	63	(72–53)	59	(66–51)	57	(66–51)	55	(61–50)	57	(63–50)
Pachycondyla group	67	(76–57)	61	(71–52)	60	(69–52)	57	(65–49)	58	(67–49)
Plectroctena group	54	(66–39)	50	(61–39)	49	(61–37)	44	(54–34)	44	(55–32)
Odontomachus group	52	(60–43)	45	(52–39)	44	(52–38)	42	(49–35)	41	(49–36)
Hypoponera	37	(47–27)	35	(47–24)	32	(43–23)	30	(39–21)	30	(40–22)
Neoponera	19	(26–14)	16	(21–13)	18	(23–13)	16	(22–10)	16	(24–12)
Leptogenys	31	(38–25)	32	(40–26)	30	(38–24)	28	(33–22)	29	(36–22)
Odontomachus + *Anochetus*	34	(42–27)	31	(35–26)	31	(37–24)	28	(33–24)	30	(33–26)

The K/T boundary (about 65 Mya: Chenet *et al.*, 2007) roughly coincides with a period of major basal radiations in Ponerinae, including the origins of crown Platythyreini (preferred age of 62 Mya), the *Ponera* group + *Pachycondyla* group (67 Mya), the MRCA of *Hypoponera* + *Plectroctena* group + *Odontomachus* group (67 Mya), the MRCA of *Hypoponera* + *Plectroctena* group (63 Mya), and the crown *Pachycondyla* (61 Mya) and *Ponera* groups (59 Mya). The origin of stem *Harpegnathos* may also be nested among these nodes. The *Plectroctena* and *Odontomachus* groups began their radiations somewhat later, around 50 and 45 Mya, respectively, with most genera in the *Odontomachus* group arising during an explosive period of diversification between 40 and 30 Mya (mid-Eocene to early Oligocene).

FIGURE 7. Dated phylogeny (maximum clade credibility tree with mean node ages) of Ponerinae inferred by BEAST analysis of the ALL_DATA matrix with a 155 Mya root age and with CAD included for *Harpegnathos*. Genus groups within Ponerini are demarcated by branch color. Clade support values and most outgroups are not shown. K/T: K/T boundary (65 Mya; vertical yellow line). The entire dated phylogeny, including outgroups, is given in Supp. Fig. S6.

Ponerine historical biogeography

A reliable inference of ponerine historical biogeography was precluded by residual uncertainty in the basal relationships within Ponerini and the existence of biogeographically-informative gaps in this study's taxon sampling. Still, strong biogeographical patterns exist for most major ponerine clades and it is possible to generalize about the major trends. The *Pachycondyla* group is entirely restricted to the Neotropics, while *Harpegnathos* is restricted to Asia. The *Ponera* group is predominantly Eurasian and Australasian in distribution, though a small number of taxa occur in the New World or Africa. The *Plectroctena* group is overwhelmingly Afrotropical, with only a few *Centromyrmex* species occurring in Asia or the Neotropics. The *Odontomachus* group exhibits a primarily Old World distribution, with roughly similar diversity in Africa/Madagascar and Asia/Australia, though many species of *Leptogenys*, *Anochetus* and *Odontomachus* occur in the New World. *Hypoponera* and *Platythyrea* are more evenly distributed among these three broad biogeographic provinces.

A cursory examination of the inferred phylogeny of Ponerinae suggests a complex biogeographic history for Ponerinae (Fig. 8), as a large number of range evolution events (vicariance or dispersal) are required to explain current ponerine distribution patterns. The ancestral distributions of both stem and crown Ponerinae are ambiguous among the three biogeographic provinces, as are the distributions of the MRCA of *Platythyrea* and of Ponerini. The MRCA of the *Ponera* and *Pachycondyla* groups most likely inhabited either the present-day Neotropics or Asia/Australia, based on the strong concentration of those clades in these regions today. Multiple faunal exchanges between the New World and Asia/Australia must have occurred in the *Ponera* group, in *Ponera*, *Cryptopone*, and *Pachycondyla* (*Pseudoponera*). An additional possible exchange in this group may have occurred in the ancestor of *Belonopelta*, a Neotropical genus which was not sampled in this study but is probably closely related to *Emeryopone* (C. Schmidt and S. Shattuck, in prep.). The *Pachycondyla* group apparently experienced a gradual but rich radiation in the Neotropics.

In contrast to the *Ponera* and *Pachycondyla* groups, most diversification in the other half of the basal split in Ponerini occurred in the Old World, with perhaps at least five independent invasions of the New World, by *Hypoponera*, *Centromyrmex*, *Leptogenys*, *Anochetus*, and *Odontomachus*. Most of the basal nodes in this large clade are ambiguous between an African/Malagasy and Asian/Australian origin, though the MRCA of the *Plectroctena* group most likely was African; the biogeographic origin of *Hypoponera* is ambiguous. The biogeographic history of the *Odontomachus* group is extremely complex, with the MRCA of the group likely either Asian/Australian or African. One side of the basal split is largely Asian (*Myopias* + *Leptogenys*), though there is much ambiguity regarding *Leptogenys*, due to the limited taxon sampling within this species-rich and geographically dispersed genus. Overall, it appears that the *Odontomachus* group underwent an explosive radiation in Africa between 40 and 30 Mya, with several subsequent invasions of Asia and the Neotropics.

The inferred divergence dates in Ponerinae are inconsistent with a major role for vicariance due to continental breakup in shaping ponerine biogeography. The basal split in Ponerinae (94 Mya in the preferred analysis) significantly postdates the purported time frame for the breakup of Africa and South America (110 Mya; Sanmartín and Ronquist, 2004), though there is little evidence of phylogenetic splits between African and Neotropical ponerine clades regardless. Faunal exchange between Africa and Asia was apparently common during the evolution of Ponerinae, especially in the *Odontomachus* group. It is not necessary to invoke long distance dispersal in this case, as Africa and Eurasia first became connected beginning around 19 Mya (Cox and Moore, 2000), consistent with the inferred ages for dispersal events between these continents. For example, *Leptogenys* is inferred to have dispersed between Asia and Africa between roughly 15 and 12 Mya, shortly after the joining of these continents. More recent dispersals have also apparently occurred between these continents, such as in *Pachycondyla* (*Brachyponera*).

The termination of a physical connection between Australia and South America apparently did not play a major role in shaping ponerine biogeography. These continents became fully separated (via Antarctica) between 52 and 35 Mya (Sanmartín and Ronqust, 2004). Few, if any, of the phylogenetic splits in Ponerinae between Asian/Australian and New World clades coincided temporally with this continental breakup. The split between the *Ponera* group (most likely with an Asian MRCA) and the *Pachycondyla* group (with a Neotropical MRCA) was inferred to have occurred significantly earlier (67 Mya), while most other such splits are younger than 35 Mya (some are very recent, such as the inferred invasions of the New World by *Ponera* and *Cryptopone*). The mechanisms and routes of dispersal between Eurasia/Australia and the New World are unclear, especially in the

case of more tropical groups such as *Centromyrmex* and *Odontomachus*. Taxa with more temperate distributions, such as *Ponera*, *Cryptopone* and *Hypoponera*, in theory could have migrated at northern latitudes between Europe or Asia and North America.

In sum, the phylogenetic and biogeographic patterns within Ponerinae suggest a history of localized radiations and frequent faunal exchange between regions. Additional targeted taxon sampling would provide a wealth of additional details about the history of ponerine range evolution, and correlation of the inferred dates of radiations and dispersal events with paleoecological data would enable future tests of hypotheses about the selective forces driving ponerine diversification.

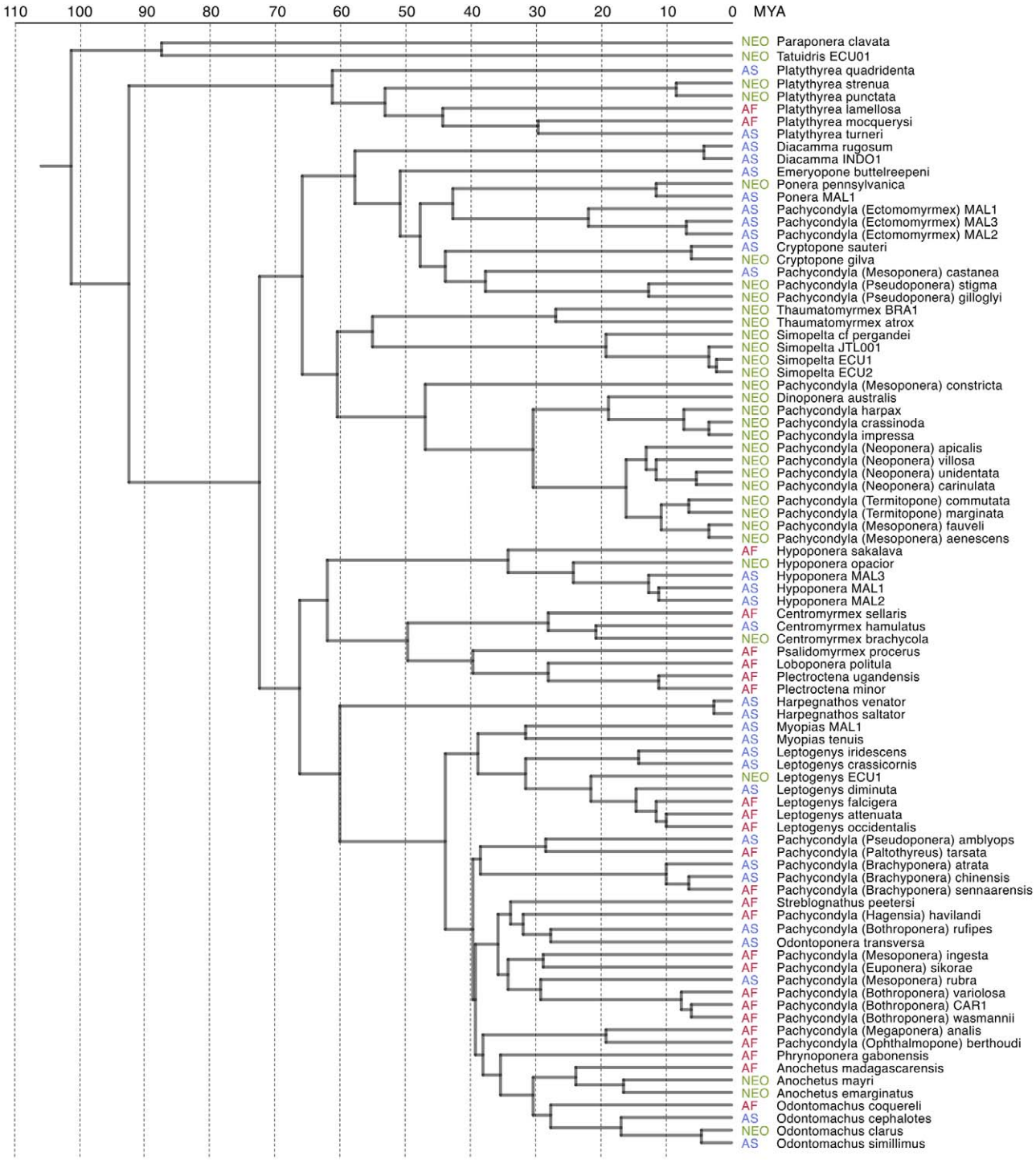


FIGURE 8. Dated phylogeny of Ponerinae showing the geographic distributions of terminal taxa. All outgroups except *Paraponera* and *Tatuidris* are excluded. “NEO”/green: New World. “AS”/blue: Asia/Australia. “AF”/red: Africa/Madagascar.

Ponerine ecological and social evolution

This study did not attempt to derive model-based estimates of ancestral states for ponerine ecological and social traits. Future studies should emphasize more focused taxon sampling, robust assessment of character states, and development of appropriate evolutionary models, before attempting to reconstruct the timing and directionality of trait evolution in a quantitative framework. While such analyses were beyond the scope of this study, and despite significant uncertainty about the basal topology of Ponerini, the inferred phylogeny does reveal much about ponerine ecological and social evolution.

Those characteristics of ponerines that are often considered primitive are indeed widespread across the phylogeny of Ponerinae (C. Schmidt and S. Shattuck, in prep.), supporting the hypothesis that these are the plesiomorphic states within the group. This is true, for example, of the following traits: solitary foraging; generalized prey preferences; relatively limited chemical communication among nestmates; a monomorphic worker caste; poor morphological differentiation between workers and queens; small colony sizes; subarboreal nesting; and simple nest construction. From this generalized condition, ponerines evolved a great wealth of forms, lifestyles and social organizations.

Ponerine ecological evolution has been marked by a diversification in prey preferences, degree of nestmate cooperation, and foraging environments. Most ponerines are generalist arthropod predators and scavengers, but a large number of genera have specialized on specific types of prey, including isopods (*Leptogenys*; Dejean and Evraerts, 1997), millipedes (*Plectroctena, Thaumatomyrmex*; Brandão, 1991; Suzzoni *et al.*, 2000), earthworms (*Psalidomyrmex*; Lévieux, 1982), other ants (*e.g.*, *Simopelta*; Gotwald and Brown, 1967), and termites (many taxa). Prey specialization in ponerines is often correlated with the presence of highly derived mandibular structure, and in fact prey specialization is probably responsible for much of the morphological diversity in Ponerinae.

Another notable aspect of ponerine foraging behavior is the degree of cooperation among foraging nestmates. This trait varies greatly among ponerine taxa, from solitary foraging without nestmate recruitment (apparently typical of most ponerines) to solitary foraging with limited recruitment (known to occur in an increasing number of ponerine taxa) to obligate collective foraging of various types and degrees. Obligate collective foraging is highly developed in *Pachycondyla* (*Megaponera*), *Pachycondyla* (*Termitopone*), and members of the *Leptogenys processionalis* (Jerdon) group, all of which are specialist mass raiders of termites and all of which inhabit different biogeographic regions (Afrotropical, Neotropical, and Indo-Australian, respectively; Wheeler, 1936; Longhurst and Howse, 1979; Dejean and Evraerts, 1997). The phylogenetic results reported here confirm that these three lineages are distantly related to one another and represent three phylogenetically and geographically independent origins of termite raiding in the Ponerinae.

Among ponerines, collective foraging is most highly developed in the Neotropical genus *Simopelta*, which has converged to a striking degree with the true army ants (subfamilies Dorylinae, Aenictinae and Ecitoninae). *Simopelta* are nomadic specialist mass raiders of other ants, have dichthadiigyne queens (ergatoid queens with enlarged gasters, exhibiting pulsed brood production), and often even have the same enlarged single-facet eyes that are present in *Eciton* (Gotwald and Brown, 1967). *Simopelta* represents yet another independent origin of mass raiding behavior in the Ponerinae from an ancestor with solitary foraging. The additional occurrence of less sophisticated cooperative foraging in numerous other ponerine lineages makes the Ponerinae an excellent model system for investigating the ecological correlates of collective foraging.

Ponerines can be broadly considered to be either epigeic or hypogeic foragers, though these categories are not entirely discrete and there are many intermediates. Workers of epigeic ponerines forage on the surface of the ground or on vegetation, have well-developed eyes, and are typically large-bodied. Workers of hypogeic (or cryptobiotic) ponerines forage in soil, rotting wood, or leaf litter, have small or absent eyes, and are usually small-bodied. Epigeic foraging is characteristic of *Platythyrea*, *Harpegnathos*, *Diacamma*, and most members of the *Odontomachus* and *Pachycondyla* groups, while cryptobiotic foraging is characteristic of *Hypoponera,* the *Plectroctena* group, most members of the *Ponera* group, and a few members of the *Odontomachus* and *Pachycondyla* groups. Taxa with intermediate foraging habits are widely spread among the *Ponera, Pachycondyla, Plectroctena,* and *Odontomachus* groups. The inferred sister group of Ponerinae is composed of one epigeic taxon (*Paraponera*) and one hypogeic taxon (*Tatuidris*).

The phylogenetic distribution of epigeic and hypogeic foraging is ambiguous regarding the ancestral condition in Ponerinae, particularly given the uncertainty in genus group relationships and the rooting of Ponerini. A formal

ancestral state analysis for this character is beyond the scope of this study, but I hypothesize that the MRCA of Ponerinae was most likely epigeic, for the following reasons: *Platythyrea*, which forms one half of the basal split in Ponerinae, is exclusively epigeic. Among the six genus groups of Ponerini, one is exclusively epigeic (*Harpegnathos*), one probably had an epigeic MRCA (the *Odontomachus* group), two have ambiguous ancestral states (the *Ponera* and *Pachycondyla* groups), and the two genus groups of Ponerini that probably had hypogeic ancestors (*Hypoponera* and the *Plectroctena* group) are most frequently inferred as sister groups and may represent a single origin of hypogeic foraging. Given these hypothesized ancestral states for ponerine genus groups, probably only a hypothetical rooting of Ponerini with *Hypoponera* and the *Plectroctena* group forming a basal grade would most parsimoniously suggest a hypogeic ancestor for Ponerinae. Though a rooting at *Hypoponera* or the *Plectroctena* group could not be rejected by an SH test (Table 7), the Bayesian results do not support these rootings. Ponerines most likely evolved cryptobiotic foraging multiple times independently.

Ponerine social behavior is a topic of intense research interest due to the high diversity of social organizations and mating systems within the subfamily. The MRCA of Ponerinae probably had a social organization that is considered plesiomorphic within ants, including small colonies and a reproductive division of labor between an alate queen caste and a non-mating monomorphic worker caste, these castes being otherwise relatively undifferentiated morphologically. From this ancestral condition, ponerines have evolved an extensive diversity of social behaviors (C. Schmidt and S. Shattuck, in prep.). Most ponerines have colonies with a few dozen to a few hundred adult individuals, but colony sizes range from half a dozen in *Thaumatomyrmex* (Jahyny *et al.*, 2002) to tens of thousands in some swarm raiding members of the *Leptogenys processionalis* group (Witte and Maschwitz, 2000).

While most ponerines have a monomorphic worker caste, several lineages have polymorphic worker castes, including *Pachycondyla* (*Megaponera*) *analis* (Latreille), *P.* (*Termitopone*) *laevigata* (Smith) and *P.* (*Termitopone*) *marginata* (Roger), *P.* (*Brachyponera*) *sennaarensis* (Mayr), and the *Centromyrmex bequaerti* (Forel) group (Wheeler, 1936; Crewe *et al.*, 1984; Dejean and Lachaud, 1994; Bolton and Fisher, 2008). The phylogenies inferred in this study suggest that each of these groups evolved its polymorphic workers independently, providing an excellent opportunity to study the evolution of worker polymorphism in a phylogenetically-informed context. Three polymorphic ponerine lineages [*P.* (*Megaponera*) *analis*, *P.* (*Termitopone*), and *Centromyrmex bequaerti*] are specialist predators of termites with at least some degree of nestmate recruitment during foraging, and all apparently have monomorphic sister taxa that are also specialist termite predators. Polymorphism in these groups may provide greater foraging efficiency relative to their monomorphic sisters, or it may enable them to prey on termite species that are too dangerous to hunt individually (Dejean and Fénéron, 1999). Worker polymorphism in *P.* (*Brachyponera*) *sennaarensis* is likely related to its granivorous habits (as in some other seed-eating ants such as *Pheidole*; Dejean and Lachaud, 1994).

Two important trends in ponerine social organization include the frequent loss of wings in the queen caste, resulting in worker-like ergatoid queens, and the occurrence of sexual reproduction by mated gamergate workers, often with the loss of the queen caste (Monnin and Peeters, 2008). Both ergatoids and gamergates are hypothesized to represent strategies for rapid and less energetically costly colony reproduction via fission, albeit with the cost of losing long-range dispersal capabilities (Molet and Peeters, 2006). The presence of ergatoids or gamergates is generally presumed to be derived, and the phylogenetic distribution of gamergate workers in Ponerinae suggests multiple independent origins of gamergates from ancestors with non-reproductive workers (C. Peeters and C. Schmidt, in prep.). Given these numerous independent origins of gamergates, Ponerinae provides a powerful tool for investigating the selection pressures favoring worker reproduction.

Conclusion

The phylogenetic relationships and inferred divergence dates within Ponerinae suggest that the ancestors of extant ponerine genera arose through a combination of fairly rapid radiations and gradual diversification over the past 100 to 90 Myr, with an early burst of radiation between 70 and 60 Mya. Care must be taken in interpreting these results, as the fossil record of Ponerinae is generally very poor, limiting our knowledge of extinct ponerine lineages. The degree to which extinction has altered the apparent shape of the ponerine phylogeny is impossible to ascertain. In addition, the taxon sampling scheme of this study emphasized genus-level taxa, leading to a somewhat skewed

view of ponerine phylogenetic history. A complete phylogeny of all extant ponerine species would be dominated by the recent radiations of the genera *Hypoponera*, *Leptogenys*, *Anochetus*, and *Odontomachus*, which together account for nearly two-thirds of described ponerine species diversity. Furthermore, the rapidity of radiations in several parts of the phylogeny could be exaggerated by the particular genes selected for this study. Sampling a range of additional genes with diverse evolutionary rates could increase topological resolution in some cases, such as at the base of Ponerini and the base of the *Odontomachus* group, and reveal a more gradual sequence of cladogenesis than is now apparent. With these caveats in mind, it is possible to begin to evaluate hypotheses about the ecological correlates of ponerine diversification.

Recent studies (Wilson and Hölldobler, 2005; Moreau *et al.*, 2006) have suggested a correlation between the radiations of ants and angiosperms. These authors hypothesized that the increased complexity of angiosperm-dominated forests, and the rich food sources represented by plants and herbivorous insects, enabled ants to radiate and attain the ecological dominance that they enjoy today. Wilson and Hölldobler (2005) further developed hypotheses to explain the diversification of Ponerinae specifically. They first implicitly assumed that the complexity of social organization in an ant lineage should be correlated with its ecological success (*i.e.*, its diversity and abundance). In this respect, Ponerinae is anomalous among ant subfamilies: ponerines tend to have relatively simple social organization but are highly diverse and abundant worldwide. Ants in other subfamilies with comparable diversity (Dolichoderinae, Formicinae and Myrmicinae) generally have much more complex social organizations (Hölldobler and Wilson, 1990). Wilson and Hölldobler labelled this "The Ponerine Paradox" and proposed two possible explanations for it. Their first hypothesis was that Ponerinae may be non-monophyletic, but this study strongly supports the subfamily's monophyly. Applying their argument to the broader, non-monophyletic assemblage of poneroid subfamilies does not change the conclusion significantly, as most species diversity among the poneroids is contained within Ponerinae *sensu stricto*. Their other explanation (the "dynastic-succession hypothesis") was that ponerines radiated prior to other dominant subfamilies and thereby co-opted the ground and leaf litter arthropod predator niche. They hypothesized that this early radiation resulted in most of the "adaptive types" in Ponerinae, with the implication that ponerines have undergone relatively little subsequent diversification in the face of competition from dominant formicoid lineages.

The results of this study support some, but not all, aspects of the dynastic-succession hypothesis. Wilson and Hölldobler suggested that Ponerinae underwent the bulk of its radiation just after the K/T boundary, during the Paleocene or early Eocene (roughly 65 to 50 Mya; International Commission on Stratigraphy, 2008) or possibly just before. This is remarkably consistent with the dates inferred here for the basal-most radiations in *Platythyrea* and Ponerini (Table 9). The present study's taxon sampling within Dolichoderinae, Myrmicinae and Formicinae is too sparse to enable robust comparisons of the relative timing of diversification within these subfamilies and Ponerinae. The reported results indicate, however, that the early radiations of Myrmicinae and Formicinae probably occurred more or less concurrently with those of Ponerinae, and the divergence dates estimated for Dolichoderinae by Ward *et al.* (2010) suggest a similarly contemporaneous early radiation in that subfamily (Supp. Fig. S6; also Moreau *et al.*, 2006, and Brady *et al.*, 2006).

In contrast to Wilson and Hölldobler's implication that relatively little of significance has happened in ponerine evolution since their initial radiation, the results reported here demonstrate that ponerines have experienced a rich history of continuous diversification over the past 50 Myr. Perhaps the most dramatic radiation of ponerine adaptive forms occurred in the *Odontomachus* group during the upper Eocene and lower Oligocene, roughly 40 to 30 Mya. This impressively rich diversification resulted in a collection of taxa so morphologically divergent from one another that some have at times been placed in their own tribes. Genus-level taxa in the other genus groups arose more gradually after the initial basal radiation of Ponerini. At the species level, major radiations have occurred in *Hypoponera* (138 described extant species, with a crown age of 35 Mya), *Leptogenys* (211 species, crown age of 32 Mya), *Anochetus* and *Odontomachus* (158 species combined, crown age of 31 Mya), and *Pachycondyla* (*Neoponera*) and its close relatives (33 species, crown age of 16 Mya).

Several factors may explain the rich evolutionary history of Ponerinae. The increased complexity of soil and leaf litter nesting microhabitats afforded by the newly-arisen angiosperm forests undoubtedly created many new niches for ponerines to fill, as has been previously suggested (Wilson and Hölldobler, 2005). The increased abundance and diversity of insect prey (Grimaldi and Engel, 2005) also likely contributed. Frequent prey specialization may be responsible for much of the group's morphological and behavioral evolution. It is possible that the diversification of termites (Isoptera) in particular could have partially driven ponerine evolution, as

termites are today a major or exclusive food source for many (perhaps even most) ponerines. The apparent timing of a major termite radiation near the K/T boundary (Thorne *et al.*, 2000; Grimaldi and Engel, 2005; Brandl *et al.*, 2007) is consistent with this hypothesis because ponerines experienced their own radiation at that time. The propensity of ponerines to experiment with novel social systems may also have driven some of their diversification. Finally, frequent dispersal between geographic regions provided numerous opportunities for ponerines to diversify.

In summary, molecular phylogenetic results suggest that a revision to the dynastic-succession hypothesis is warranted. Ponerines and myrmicines came to dominate the ground and leaf litter strata of tropical forests, while the formicines and dolichoderines came to dominate the arboreal strata, with some overlap among these niche specializations. Rather than a sequential series of radiations, first in Ponerinae, then in Myrmicinae, and lastly in Formicinae and Dolichoderinae, it appears more likely that these four dominant ant subfamilies diversified together. If true, this hypothesis poses a new, more challenging question: Why did Ponerinae achieve pantropical ecological dominance instead of other ant subfamilies of comparable age with similar suites of social, behavioral, and morphological characteristics, such as Amblyoponinae or Proceratiinae?

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SUPPLEMENTAL TABLE S1. List of sampled ingroup taxa (Formicidae, Ponerinae), with CASENT (California Academy of Sciences) accession numbers for morphological and DNA voucher specimens and GenBank accession numbers for DNA sequences. Taxa marked with an "*" are members of the "Additional Ingroup" taxon set; all other listed taxa are considered members of the "Core Ingroup" taxon set. "n/a": Collection locality not available.

Tribe	Genus	Species	Authority	Locality	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Platythyreini	*Platythyrea*	*lamellosa*	(Roger)	South Africa	0260200	JN419121	JN675407	JX310642	JQ023503	-	-	-
Platythyreini	*Platythyrea*	*mocquerysi**	Emery	Madagascar	1060941	AY867422[1]	AY867484[1]	-	AY867453[1]	AY867469[1]	EF013322[1]	EF013484[1]
Platythyreini	*Platythyrea*	*punctata*	(Smith)	USA	0260201, 0006819[1]	JN419122	EF013620[1]	JX310643	JQ023504	EF013168[1]	EF013323[1]	EF013485[1]
Platythyreini	*Platythyrea*	*quadridenta*	Donisthorpe	Malaysia	0260202	JN419123	JN675408	JX310644	-	-	-	-
Platythyreini	*Platythyrea*	*strenua*	Wheeler & Mann	Dominican Republic	0260203	EU155479[4]	EU155460[4]	JX310645	EU155423[4]	-	-	-
Platythyreini	*Platythyrea*	*turneri*	Forel	Australia	0260204	JN419124	-	-	-	-	-	-
Ponerini	*Anochetus*	*emarginatus*	(Fabricius)	Trinidad	0260205	EU155462[4]	EU155443[4]	JX310572	JQ023505	-	-	-
Ponerini	*Anochetus*	*madagascarensis*	Forel	Madagascar	0260206, 0498593[1]	JN419125	EF013542[1]	JX310573	EF012962[1]	EF013090[1]	EF013221[1]	EF013383[1]
Ponerini	*Anochetus*	*mayri*	Emery	Trinidad	0260207	JN419126	JN675409	JX310574	JQ023506	-	-	-
Ponerini	*Centromyrmex*	*brachycola*	(Roger)	Trinidad	0260208	JN419127	JN675410	JX310575	JQ023507	-	-	-
Ponerini	*Centromyrmex*	*hamulatus*	(Karavaiev)	Malaysia	0260209	JN419128	JN675411	JX310576	JQ023508	-	-	-
Ponerini	*Centromyrmex*	*sellaris**	Mayr	n/a	4171471	EF013687[1]	EF013559[1]	-	EF012979[1]	EF013107[1]	EF013241[1]	EF013403[1]
Ponerini	*Cryptopone*	*gilva*	(Roger)	USA	0260210	JN419129	JN675412	JX310578	JQ023509	-	-	-
Ponerini	*Cryptopone*	*sauteri*	(Wheeler)	Japan	0260211	JN419130	JN675413	JX310579	JQ023510	-	-	-
Ponerini	*Diacamma*	*rugosum*	(Le Guillou)	Indonesia	0260212	JN419131	JN675414	JX310580	-	-	-	-
Ponerini	*Diacamma*	sp. Indo1	-	Indonesia	0260213	JN419132	JN675415	JX310581	JQ023511	-	-	-
Ponerini	*Dinoponera*	*australis*	Emery	Argentina	0260214	JN419133	JN675416	JX310582	-	-	-	-
Ponerini	*Emeryopone*	*buttelreepeni*	Forel	Malaysia	0260215	JN419134	JN675417	JX310583	JQ023512	-	-	-
Ponerini	*Harpegnathos*	*saltator*	Jerdon	India	0260216	JN419135	JN675418	JX310584	JQ023513	-	-	-

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SUPPLEMENTAL TABLE S1. (Continued)

Tribe	Genus	Species	Authority	Locality	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Ponerini	*Harpegnathos*	*venator*	(Smith)	Malaysia	0260217	JN419136	JN675419	JX310585	JQ023514	-	-	-
Ponerini	*Hypoponera*	*opacior*	(Forel)	USA	0260218, 0106093[1]	EU155464[4]	EU155445[4]	JX310587	EU155410[4]	AY703689[1]	EF013266[1]	EF013428[1]
Ponerini	*Hypoponera*	*sakalava*	(Forel)	Madagascar	0260219, 0494141[1]	JN419137	EF013577[1]	JX310588	JQ023515	EF013125[1]	EF013267[1]	EF013429[1]
Ponerini	*Hypoponera*	sp. Mal1	-	Malaysia	0260220	JN419138	n/a[6]	JX310589	JQ023516	-	-	-
Ponerini	*Hypoponera*	sp. Mal2	-	Malaysia	0260221	JN419139	JN675420	JX310590	JQ023517	-	-	-
Ponerini	*Hypoponera*	sp. Mal3	-	Malaysia	0260222	JN419140	JN675421, JN675422	JX310591	JQ023518	-	-	-
Ponerini	*Leptogenys*	*attenuata*	(Smith)	South Africa	0260223	EU155465[4]	EU155446[4]	JX310592	EU155411[4]	-	-	-
Ponerini	*Leptogenys*	*crassicornis*	Emery	Malaysia	0260224	JN419145	JN675423	JX310593	JQ023522	-	-	-
Ponerini	*Leptogenys*	*diminuta**	(Smith)	n/a	1060101	EF013708[1]	EF013580[1]	-	EF013000[1]	EF013128[1]	EF013273[1]	EF013435[1]
Ponerini	*Leptogenys*	*falcigera*	Roger	USA (Hawaii)	0260225	JN419142	JN675424	JX310594	JQ023519	-	-	-
Ponerini	*Leptogenys*	*iridescens*	(Smith)	Malaysia	0260226	JN419141	KC006064	JX310595	-	-	-	-
Ponerini	*Leptogenys*	*occidentalis*	Bernard	CAR	0260227	JN419143	JN675425	JX310596	JQ023520	-	-	-
Ponerini	*Leptogenys*	*sp. Ecu1*	-	Ecuador	0260228	JN419144	JN675426	JX310597	JQ023521	-	-	-
Ponerini	*Loboponera*	*politula*	Bolton & Brown	Cameroon	0260229, 0003095[1]	JN419146	JN675427	JX310598	JQ023523	EF013133[1]	EF013280[1]	EF013442[1]
Ponerini	*Myopias*	sp. Mal1	-	Malaysia	0260230	JN419147	JN675428	JX310599	JQ023524	-	-	-
Ponerini	*Myopias*	*tenuis*	(Emery)	Australia	0260231	JN419148	JN675429	JX310600	JQ023525	-	-	-
Ponerini	*Odontomachus*	*cephalotes*	Smith	Australia	0260232	EU155468[4]	EU155449[4]	JX310604	EU155413[4]	-	-	-
Ponerini	*Odontomachus*	*clarus*	Roger	USA	0260233	EU155479[4]	EU155451[4]	JX310605	EU155414[4]	-	-	-
Ponerini	*Odontomachus*	*coquereli**	Roger	Madagascar	4995251	EF013734[1]	EF013606[1]	-	EF013026[1]	EF013154[1]	EF013307[1]	EF013469[1]
Ponerini	*Odontomachus*	*simillimus*	Smith	Indonesia	0260234	JN419149	JN675430	JX310606	JQ023526	-	-	-
Ponerini	*Odontoponera*	*transversa*	(Smith)	Indonesia	0260235, 0010127[1]	EU155478[4]	EU155459[4]	JX310607	EU155422[4]	EF013155[1]	EF013308[1]	EF013470[1]
Ponerini	*Pachycondyla (Pachycondyla)*	*crassinoda*	(Latreille)	Peru	0260236	JN419158	JN675442	JX310619	JQ023533	-	-	-

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SUPPLEMENTAL TABLE S1. (Continued)

Tribe	Genus	Species	Authority	Locality	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Ponerini	*Pachycondyla (Pachycondyla)*	*harpax*	(Fabricius)	Trinidad	0260237	JN419163	JN675444	JX310621	JQ023538	-	-	-
Ponerini	*Pachycondyla (Pachycondyla)*	*impressa*	(Roger)	Panama	0260238	JN419164	JN675445	JX310623	JQ023539	-	-	-
Ponerini	*Pachycondyla (Bothroponera)*	*rufipes*	(Jerdon)	India	0260239	JN419151	JN675449	JX310627	JQ023527	-	-	-
Ponerini	*Pachycondyla (Bothroponera)*	sp. CAR1	-	CAR	0260240	JN419152	JN675451	JX310630	JQ023528	-	-	-
Ponerini	*Pachycondyla (Bothroponera)*	*variolosa*	(Arnold)	South Africa	0260241	JN419154	JN675459	JX310638	JQ023530	-	-	-
Ponerini	*Pachycondyla (Bothroponera)*	*wasmannii*	(Forel)	Madagascar	0260242	JN419153	JN675461	JX310640	JQ023529	-	-	-
Ponerini	*Pachycondyla (Brachyponera)*	*atrata*	(Karavaiev)	Indonesia	0260243	JN419155	JN675435	JX310612	JQ023531	-	-	-
Ponerini	*Pachycondyla (Brachyponera)*	*chinensis*	(Emery)	USA	0260244	JN419156	JN675439	JX310616	AB126802[2]	-	-	-
Ponerini	*Pachycondyla (Brachyponera)*	*sennaarensis*	(Mayr)	Qatar	0260245	JN419157	JN675450	JX310628	JQ023532	-	-	-
Ponerini	*Pachycondyla (Ectomomyrmex)*	sp. Mal1	-	Malaysia	0260246	JN419159	JN675453	JX310632	JQ023534	-	-	-
Ponerini	*Pachycondyla (Ectomomyrmex)*	sp. Mal2	-	Malaysia	0260247	JN419160	JN675454	JX310633	JQ023535	-	-	-
Ponerini	*Pachycondyla (Ectomomyrmex)*	sp. Mal3	-	Malaysia	0260248	JN419161	JN675455	JX310634	JQ023536	-	-	-
Ponerini	*Pachycondyla (Euponera)*	*sikorae*	(Forel)	Madagascar	0260249, 0487847[1]	JN419150	EF013612[1]	JX310629	EF013032[1]	EF013160[1]	EF013313[1]	EF013475[1]
Ponerini	*Pachycondyla (Hagensia)*	*havilandi*	Forel	South Africa	0260250	JN419162	-	JX310622	JQ023537	-	-	-
Ponerini	*Pachycondyla (Megaponera)*	*analis*	(Latreille)	Sudan	0260251	JN419165	JN675433	JX310610	JQ023540	-	-	-
Ponerini	*Pachycondyla (Mesoponera)*	*aenescens*	Mayr	Costa Rica	0260252	JN419166	JN675431	JX310608	JQ023541	-	-	-

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SUPPLEMENTAL TABLE S1. (Continued)

Tribe	Genus	Species	Authority	Locality	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Ponerini	*Pachycondyla (Mesoponera)*	*castanea*	(Mayr)	New Zealand	0260253	JN419170	JN675438	JX310615	JQ023543	-	-	-
Ponerini	*Pachycondyla (Mesoponera)*	*constricta*	(Mayr)	Venezuela	0260254	JN419167	JN675441	JX310618	JQ023542	-	-	-
Ponerini	*Pachycondyla (Mesoponera)*	*fauveli*	Emery	Peru	0260255	JN419180	JN675443	JX310620	JQ023546	-	-	-
Ponerini	*Pachycondyla (Mesoponera)*	*ingesta*	(Wheeler)	CAR	0260255	JN419168	JN675446	JX310624	-	-	-	-
Ponerini	*Pachycondyla (Mesoponera)*	*rubra*	(Smith)	Malaysia	0260256	JN419169	JN675448	JX310626	-	-	-	-
Ponerini	*Pachycondyla (Neoponera)*	*apicalis*	(Smith)	Costa Rica	0260257	JN419171	JN675434	JX310611	JQ023544	-	-	-
Ponerini	*Pachycondyla (Neoponera)*	*carinulata*	(Roger)	Ecuador	0260258	JN419172	JN675437	JX310614	JQ023545	-	-	-
Ponerini	*Pachycondyla (Neoponera)*	*unidentata*	Mayr	Trinidad	0260259	JN419173	JN675458	JX310637	JQ023547	-	-	-
Ponerini	*Pachycondyla (Neoponera)*	*villosa*	(Fabricius)	Brazil	0260260	JN419174	JN675460	JX310639	JQ023548	-	-	-
Ponerini	*Pachycondyla (Ophthalmopone)*	*berthoudi*	(Forel)	South Africa	0260261	JN419175	JN675436	JX310613	-	-	-	-
Ponerini	*Pachycondyla (Paltothyreus)*	*tarsata*	(Fabricius)	South Africa	0260262	JN419176	JN675457	JX310636	-	-	-	-
Ponerini	*Pachycondyla (Pseudoponera)*	*amblyops*	(Emery)	Malaysia	0260263	JN419178	JN675432	JX310609	-	-	-	-
Ponerini	*Pachycondyla (Pseudoponera)*	*gilloglyi*	-	Costa Rica	0260264	JN419177	JN675452	JX310631	JQ023549	-	-	-
Ponerini	*Pachycondyla (Pseudoponera)*	*stigma*	(Fabricius)	Dominican Republic	0260265	JN419179	JN675456	JX310635	DQ353617[3]	-	-	-
Ponerini	*Pachycondyla (Termitopone)*	*commutata*	(Roger)	Venezuela	0260266	JN419181	JN675440	JX310617	JQ023550	-	-	-
Ponerini	*Pachycondyla (Termitopone)*	*marginata*	(Roger)	Paraguay	0260267	JN419182	JN675447	JX310625	JQ023551	-	-	-
Ponerini	*Phrynoponera*	*gabonensis*	(André)	CAR	0260268	JN419183	JN675462	JX310641	JQ023552	-	-	-

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SUPPLEMENTAL TABLE S1. (Continued)

Tribe	Genus	Species	Authority	Locality	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Ponerini	*Plectroctena*	*minor*	Emery	Gabon	0260269	JN419184	JN675463	JX310646	-	-	-	-
Ponerini	*Plectroctena*	*ugandensis*	Menozzi	Gabon	0260270, 0003063[1]	EU155480[4]	EU155461[4]	JX310647	EU155424[4]	EF013169[1]	EF013324[1]	EF013486[1]
Ponerini	*Ponera*	*pennsylvanica*	Buckley	USA	0260271	JN419185	JN675464	-	JQ023553	-	-	-
Ponerini	*Ponera*	sp. Mal1	-	Malaysia	0260272	JN419186	JN675465	JX310648	JQ023554	-	-	-
Ponerini	*Psalidomyrmex*	*procerus*	Emery	Gabon	0260273, 0003082[1]	JN419187	JN675466	JX310649	JQ023555	EF013182[1]	EF013338[1]	EF013500[1]
Ponerini	*Simopelta*	cf. *pergandei**	-	n/a	0527441	EF013769[1]	EF013641[1]	-	EF013061[1]	EF013189[1]	EF013346[1]	EF013508[1]
Ponerini	*Simopelta*	sp. JTL001	-	Costa Rica	0260274	JN419188	JN675469	JX310652	JQ023556	-	-	-
Ponerini	*Simopelta*	sp. Ecu1	-	Ecuador	0260275	JN419189	JN675467	JX310650	JQ023557	-	-	-
Ponerini	*Simopelta*	sp. Ecu2	-	Ecuador	0260276	JN419190	JN675468	JX310651	JQ023558	-	-	-
Ponerini	*Streblognathus*	*peetersi*	Robertson	South Africa	0260277	JN419191	JN675470	JX310653	JQ023559	-	-	-
Thaumatomyrmecini	*Thaumatomyrmex*	*atrox**	Weber	n/a	0101211	EF013782[1]	EF013654[1]	-	EF013074[1]	EF013202[1]	EF013363[1]	EF013525[1]
Thaumatomyrmecini	*Thaumatomyrmex*	sp. Bra1	-	Brazil	0260278[5]	JN419192	-	JX310654	-	-	-	-

1 Data from Brady *et al.* (2006).
2 Data from Ohnishi *et al.* (2003).
3 Data from Moreau *et al.* (2006).
4 Data from Spagna *et al.* (2008).
5 Molecular (DNA) voucher only.
6 Partial fragment, too short for GenBank submission; sequence is available from TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13082).

SUPPLEMENTAL TABLE S2. List of sampled outgroup taxa, including CASENT accessions numbers for morphological and DNA voucher specimens and GenBank accession numbers for DNA sequences. All outgroup data are from Brady *et al*. (2006) except the sequences for *Martialis heureka* (Rabeling et al., 2008), CAD for *Apis mellifera* (Danforth *et al*., 2006), and CAD for *Mystrium, Cheliomyrmex, Myrmelachista, Heteroponera,* and *Myrmicaria* (S. Brady and P.S. Ward, pers. comm.).

Family	Subfamily	Genus	Species	Authority	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Formicidae	Aenictinae	*Aenictus*	*ceylonicus*	(Mayr)	0106017	EF013666	EF013538	-	EF012958	EF013086	EF013215	EF013377
Formicidae	Aenictogitoninae	*Aenictogiton*	*ZAM02*	-	0106126	EF013665	EF013537	-	EF012957	EF013085	EF013214	EF013376
Formicidae	Agroecomyrmecinae	*Tatuidris*	*ECU01*	-	0423526	EF013775	EF013647	-	EF013067	EF013195	EF013354	EF013516
Formicidae	Amblyoponinae	*Apomyrma*	*stygia*	Brown, Gotwald & Lévieux	0007017	EF013675	EF013547	-	EF012967	EF013095	EF013228	EF013390
Formicidae	Amblyoponinae	*Concoctio*	*concenta*	Brown	0004306	EF013691	EF013563	-	EF012983	EF013111	EF013248	EF013410
Formicidae	Ambyloponinae	*Mystrium*	*mysticum*	Roger	0076622	EF013730	EF013602	JX310603	EF013022	EF013150	EF013301	EF013463
Formicidae	Ambyloponinae	*Onychomyrmex*	*hedleyi*	Emery	0106018	EF013737	EF013609	-	EF013029	EF013157	EF013310	EF013472
Formicidae	Amblyoponinae	*Prionopelta*	*MAD01*	-	0494610	EF013756	EF013628	-	EF013048	EF013176	EF013331	EF013493
Formicidae	Aneuretinae	*Aneuretus*	*simoni*	Emery	0007014	EF013669	EF013541	-	EF012961	EF013089	EF013220	EF013382
Formicidae	Cerapachyinae	*Cylindromyrmex*	*striatus*	Mayr	0106074	AY867426	AY867488	-	AY867457	AY867473	EF013250	EF013412
Formicidae	Dolichoderinae	*Anonychomyrma*	*gilberti*	(Forel)	0106003	EF013671	EF013543	-	EF012963	EF013091	EF013222	EF013384
Formicidae	Dolichoderinae	*Leptomyrmex*	*erythrocephalus*	(Fabricius)	0106077	AY703628	AY703762	-	AY703561	AY703695	EF013275	EF013437
Formicidae	Dolichoderinae	*Tapinoma*	*sessile*	(Say)	0106028	EF013774	EF013646	-	EF013066	EF013194	EF013353	EF013515
Formicidae	Dorylinae	*Dorylus*	*laevigatus*	(Smith)	0010126	EF013697	EF013569	-	EF012989	EF013117	EF013256	EF013416
Formicidae	Ecitoninae	*Cheliomyrmex*	*morosus cf*	-	0007006	EF013690	EF013562	JX310577	EF012982	EF013110	EF013246	EF013408
Formicidae	Ecitoninae	*Neivamyrmex*	*nigrescens*	(Cresson)	0106080	AY867430	AY867492	-	AY867461	AY867477	EF013302	EF013464

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SUPPLEMENTALE TABLE S2. (Continued)

Family	Subfamily	Genus	Species	Authority	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Formicidae	Ectatomminae	*Ectatomma*	*opaciventre*	(Roger)	0106061	AY703626	AY703760	-	AY703559	AY703693	EF013259	EF013421
Formicidae	Ectatomminae	*Rhytidoponera*	*chalybaea*	Emery	0106000	EF013766	EF013638	-	EF013058	EF013186	EF013343	EF013505
Formicidae	Formicinae	*Acropyga*	*acutiventris*	Roger	0106009	EF013663	EF013535	-	EF012955	EF013083	EF013212	EF013374
Formicidae	Formicinae	*Anoplolepis*	*gracilipes*	(Smith)	0106057	EF013672	EF013544	-	EF012964	EF013092	EF013223	EF013385
Formicidae	Formicinae	*Camponotus*	*conithorax*	Emery	0106008	EF013683	EF013555	-	EF012975	EF013103	EF013236	EF013398
Formicidae	Formicinae	*Myrmelachista*	*flavocotea*	Longino	0106049	EF013725	EF013597	JX310601	EF013017	EF013145	EF013295	EF013457
Formicidae	Formicinae	*Oecophylla*	*smaragdina*	(Fabricius)	0106113	EF013736	EF013608	-	EF013028	EF013156	EF013309	EF013471
Formicidae	Heteroponerinae	*Heteroponera*	*panamensis*	(Forel)	0106021	EF013704	EF013576	JX310586	EF012996	EF013124	EF013265	EF013427
Formicidae	Leptanillinae	*Leptanilla*	GRE01	-	0006814	EF013707	EF013579	-	EF012999	EF013127	EF013269	EF013431
Formicidae	Leptanillinae	*Protanilla*	JAP01	-	0007002	EF013761	EF013633	-	EF013053	EF013181	EF013337	EF013499
Formicidae	Leptanilloidinae	*Leptanilloides*	*nomada*	Donoso, Vieira & Wild	0106087	AY867428	AY867490	-	AY867459	AY867475	EF013272	EF013434
Formicidae	Martialinae	*Martialis*	*heureka*	Rabeling & Verhaagh	0106181	-	-	-	EU913473	-	-	EU913474
Formicidae	Myrmeciinae	*Nothomyrmecia*	*macrops*	Clark	0106089	AY703635	AY703769	-	AY703568	AY703702	EF013304	EF013466
Formicidae	Myrmicinae	*Acromyrmex*	*versicolor*	(Pergande)	0106056	EF013662	EF013534	-	EF012954	EF013082	EF013211	EF013373
Formicidae	Myrmicinae	*Crematogaster*	*emeryana*	Creighton	0106034	EF013692	EF013564	-	EF012984	EF013112	EF013249	EF013411
Formicidae	Myrmicinae	*Manica*	*bradleyi*	(Wheeler)	0106022	EF013714	EF013586	-	EF013006	EF013134	EF013281	EF013443
Formicidae	Myrmicinae	*Myrmica*	*tahoensis*	Weber	0106091	AY703629	AY703763	-	AY703562	AY703696	EF013297	EF013459
Formicidae	Myrmicinae	*Myrmicaria*	*exigua*	André	0403455	EF013727	EF013599	JX310602	EF013019	EF013147	EF013298	EF013460
Formicidae	Myrmicinae	*Pheidole*	*hyatti*	Emery	0106046	EF013744	EF013616	-	EF013036	EF013164	EF013318	EF013480
Formicidae	Myrmicinae	*Pheidologeton*	*affinis*	(Jerdon)	0106016	EF013745	EF013617	-	EF013037	EF013165	EF013319	EF013481
Formicidae	Myrmicinae	*Tetramorium*	*caespitum*	(Linnaeus)	0106026	EF013780	EF013652	-	EF013072	EF013200	EF013359	EF013521

……continued on the next page

SUPPLEMENTAL TABLE S2. (CONTINUED)

Family	Subfamily	Genus	Species	Authority	CASENT	Wg	LWR	CAD	28S	Abd-A	EF1αF1	EF1αF2
Formicidae	Myrmicinae	*Trachymyrmex*	*arizonensis*	(Wheeler)	0106047	EF013783	EF013655	-	EF013075	EF013203	EF013364	EF013526
Formicidae	Myrmicinae	*Vollenhovia*	*emeryi*	Wheeler	0010125	EF013785	EF013657	-	EF013077	EF013205	EF013367	EF013529
Formicidae	Paraponerinae	*Paraponera*	*clavata*	(Fabricius)	0106092	AY703623	AY703757	-	AY703556	AY703690	EF013315	EF013477
Formicidae	Proceratiinae	*Discothyrea*	MAD07	-	0042927	EF013695	EF013567	-	EF012987	EF013115	EF013253	EF013415
Formicidae	Proceratiinae	*Probolomyrmex*	*tani*	Fisher	0041507	EF013758	EF013630	-	EF013050	EF013178	EF013333	EF013495
Formicidae	Proceratiinae	*Proceratium*	*stictum*	Brown	0106095	AY703624	AY703758	-	AY703557	AY703691	EF013335	EF013497
Formicidae	Pseudomyrmecinae	*Pseudomyrmex*	*gracilis*	(Fabricius)	0106097	AY703663	AY703797	-	AY703596	AY703730	EF013340	EF013502
Formicidae	Pseudomyrmecinae	*Tetraponera*	*punctulata*	Smith	0106098	AY703648	AY703782	-	AY703581	AY703715	EF013361	EF013523
Apidae	Apinae	*Apis*	*mellifera*	Linnaeus	0106100	AY703618	AY703752	DQ067178	AY703551	AY703685	EF013227	EF013389
Bethylidae	Pristocerinae	*Pristocera*	MAD01	-	0006958	EF013757	EF013629	-	EF013049	EF013177	EF013332	EF013494
Bradynobaenidae	Chyphotinae	*Chyphotes*	*mellipes*	(Blake)	0106101	AY703619	AY703753	-	AY703552	AY703686	EF013247	EF013409
Mutillidae	Sphaerophthalminae	*Dasymutilla*	*aureola*	(Cresson)	0106123	EF013694	EF013566	-	EF012986	EF013114	EF013252	EF013414
Pompilidae	Pompilinae	*Aporus*	*niger*	(Cresson)	0106104	EF013676	EF013548	-	EF012968	EF013096	EF013229	EF013391
Sphecidae	Sceliphrinae	*Chalybion*	*californicum*	(Saussure)	0106103	EF013689	EF013561	-	EF012981	EF013109	EF013245	EF013407
Tiphiidae	Brachycistidinae	*Aglyptacros*	*cf. sulcatus*	-	0106122	EF013667	EF013539	-	EF012959	EF013087	EF013217	EF013379

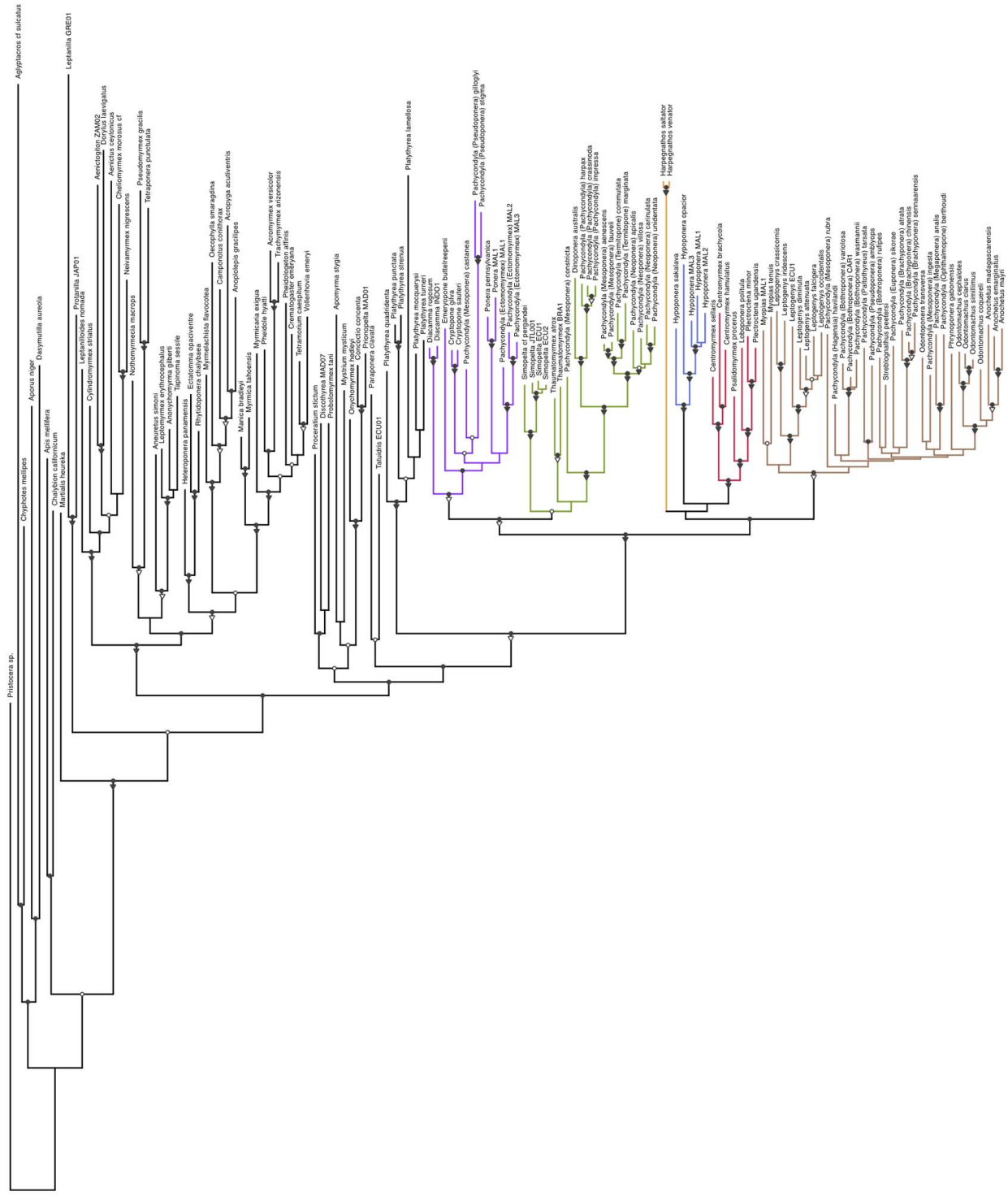
SUPPLEMENTAL TABLE S3.

Taxon	Minimum Age (Myr)	Fossil Justification
Apoidea	140	Angarosphecidae (Apoidea) in Yixian Formation (China) and Lulworth Formation (England)[1]
Aporus	100	*Bryopompilus* (Pompilidae) in Myanmar amber[2]
Chalybion	100	Crabronidae in Myanmar amber[3]
Aglyptacros	90	*Architiphia* (Tiphiidae) in Santana Formation (Brazil)[4]
Formicinae	88.6	*Kyromyrma* in New Jersey amber[5]
Dolichoderinae	60	*Eotapinoma* in Sakhalin amber[6]
Myrmeciinae	54.5	*Ypresiomyrma* in Ølst Formation (Denmark)[7]
Agroecomyrmecinae	44.1	*Agroecomyrmex* in Baltic amber[8]
Camponotus	44.1	*Camponotus* in Baltic amber[9]
Hypoponera	44.1	*Hypoponera* in Baltic amber[9]
Rhytidoponera	44.1	*Rhytidoponera* in Baltic amber[9]
Tetramorium	44.1	*Tetramorium* in Baltic amber[9]
Tetraponera	44.1	*Tetraponera* in Baltic amber[9]
Proceratiinae	44.1	*Bradoponera* in Baltic amber[10]
Cryptopone + *Pachycondyla* (*Pseudoponera*)	44.1	*"Pseudoponera"* in Baltic amber[11]
Pheidole	34	*Pheidole* in Florissant Formation[12]
Discothyrea	22.5	*Discothyrea* in Mexican amber[13]
Acropyga	15	*Acropyga* in Dominican amber[14]
Anochetus	15	*Anochetus* in Dominican amber[15]
Odontomachus	15	*Odontomachus* in Dominican amber[15]
Neivamyrmex	15	*Neivamyrmex* in Dominican amber[16]
Prionopelta	15	*Prionopelta* in Dominican amber[17]
Trachymyrmex	15	*Trachymyrmex* in Dominican amber[17]

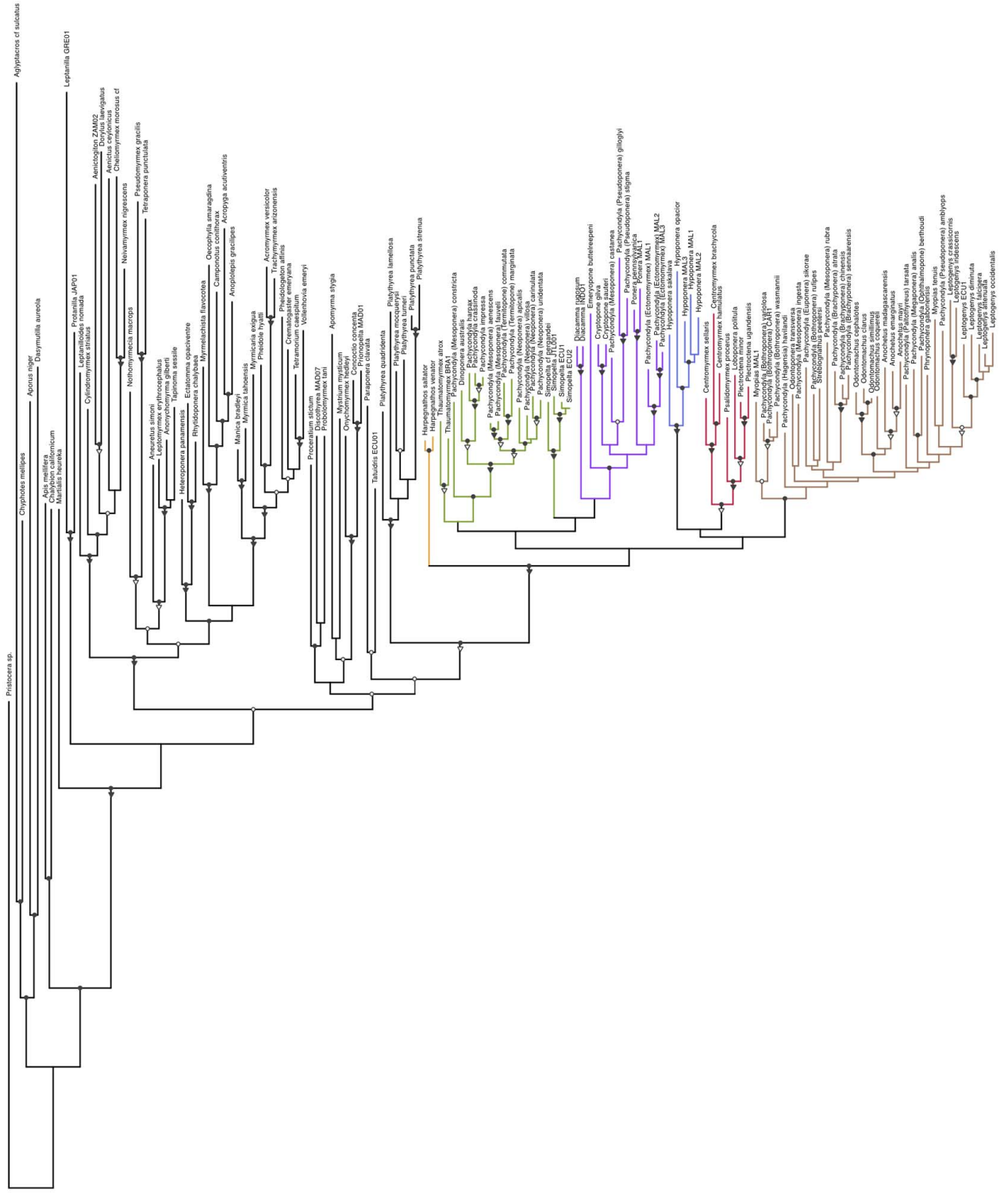
Notes About Fossil Constraints

1. Fossils described by Zhang *et al.* (2002) and Rasnitsyn *et al.* (1998). The age of the Yixian Formation is disputed, but the most recent study (Yang *et al.*, 2007) had as its youngest estimate 121.2 Mya. The Lulworth Formation is considered to be Berriasian, which ended around 140.2 Mya according to McArthur *et al.* (2007). Hence I will keep the 140 Mya age applied to this node by Brady *et al.* (2006).
2. Fossil described by Grimaldi and Engel (2006). Myanmar amber is considered to be about 100 Myr in age (Cruikshank and Ko, 2003).
3. Fossil described by Antropov (2000).
4. Fossil described by Darling and Sharkey (1990). Martill (2007) reviewed the evidence on the age of the Santana Formation and concluded that we simply don't know how old it is; cited estimates ranged from about 90 to 125 Mya. Grimaldi and Engel (2005) give an age of 120 Mya (the age used by Brady *et al.*, 2006), and Darling and Sharkey (1990) give the age of *Architiphia* as 105 Mya, but I am conservatively using the younger 90 Mya age estimate for the Santana Formation.
5. Fossil described by Grimaldi and Agosti (2000). New Jersey amber is considered to be Turonian in age (Grimaldi and Engel, 2005). I am using the upper terminus of that age, 88.6 Mya (International Commission on Stratigraphy, 2008), rather than the less conservative 92 Mya age cited by Grimaldi and Agosti (2000).
6. Fossil described by Dlussky (1988). Sakhalin amber is considered to be Paleocene in age (55.8–65.5 Mya; International Commision on Stratigraphy, 2008). *Eotapinoma* has also been described from Canadian amber (Dlussky, 1999), which is considerably older (perhaps as old as 79 Mya; Eberth and Hamblin, 1993). Given the considerable age of this latter fossil and its more obscured features, I am following Brady *et al.* (2006) in exercising caution in assigning it to Dolichoderinae, and am using the 60 Mya age they chose for the Sakhalin amber (see also Moreau *et al.*, 2006).
7. Fossil described by Archibald *et al.* (2006). The Ølst Formation is dated at roughly 54.5 Mya (Chambers *et al.*, 2003; Archibald *et al.*, 2006).

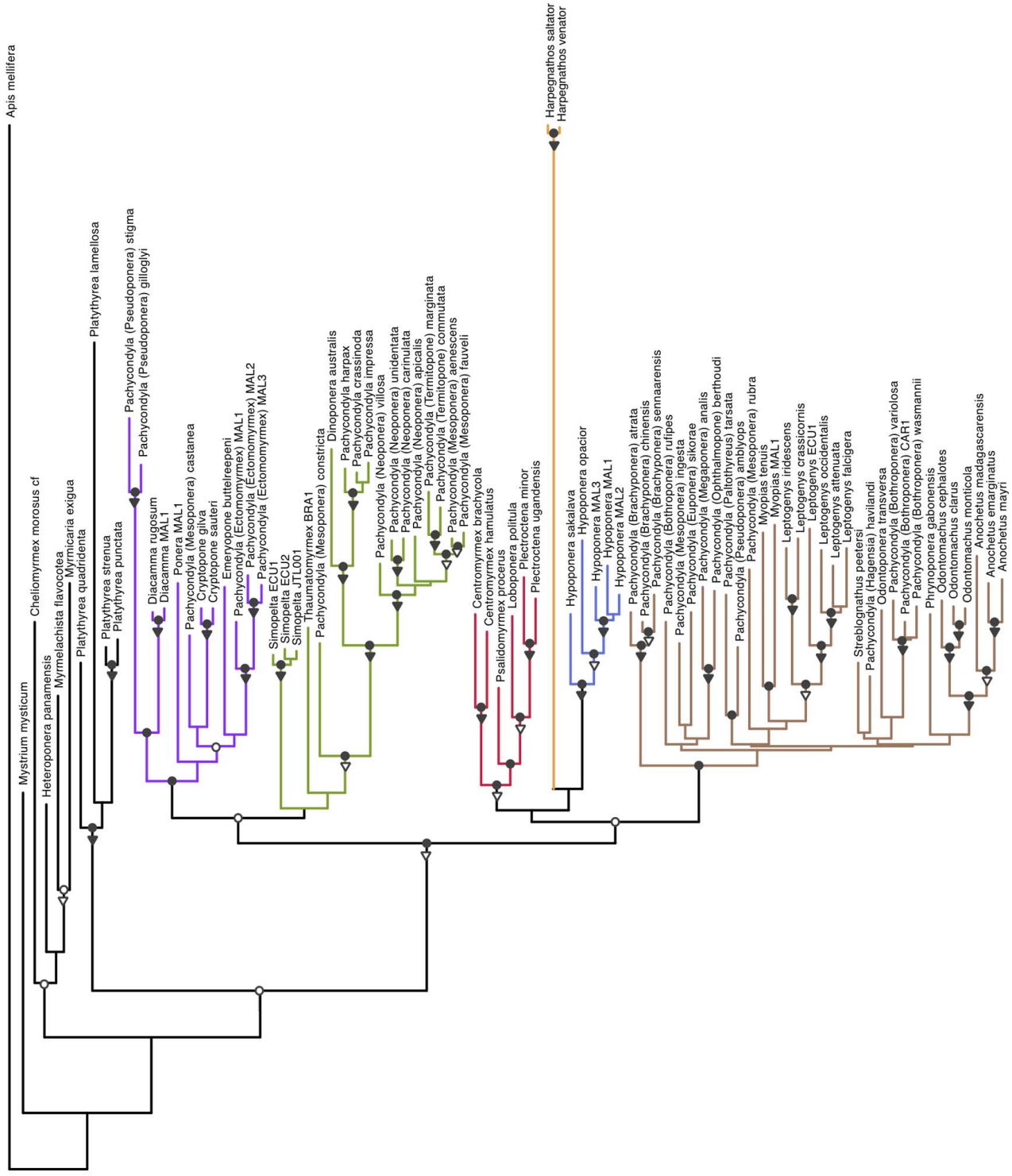
8. Fossil described by Wheeler (1915). Baltic amber is currently considered to be roughly 44 Myr old (Ritzkowski, 1997; Grimaldi and Engel, 2005), though it may be younger (discussed by Archibald *et al.*, 2006). I am following Moreau *et al.* (2006) and Brady *et al.* (2006) in using the 44.1 Mya age for Baltic amber.
9. Fossil cited by Dlussky (1997).
10. Fossil described by de Andrade and Baroni Urbani (2003).
11. Wheeler (1915) placed this fossil species (*Pachycondyla succinea* [Mayr]) in what is now *Pachycondyla* (*Pseudoponera*). From his description and drawings it does indeed appear to be at least closely related to *P.* (*Pseudoponera*), though the presence of basal mandibular pits suggests the possibility of a closer affinity to *Cryptopone*, the apparent sister group to *P.* (*Pseudoponera*). Given this uncertainty I am conservatively applying this fossil to the stem group containing both *Cryptopone* and *P.* (*Pseudoponera*).
12. Fossil described by Carpenter (1930). The Florissant Formation of Colorado is estimated to be about 34 Myr old (Evanoff *et al.*, 2001).
13. Fossil described by de Andrade (1998). Tonidandel *et al.* (2008) give the age of Mexican amber as 22.5–26 Mya, and I am conservatively using the younger estimate of 22.5 Mya.
14. Fossil described by LaPolla (2005). The age of Dominican amber is estimated at 15-20 Mya (Iturralde-Vinent and MacPhee, 1996), and I am conservatively using the younger age of 15 Mya.
15. Fossil described by de Andrade (1994).
16. Fossil described by Wilson (1985).
17. Fossil cited by Wilson (1985B).



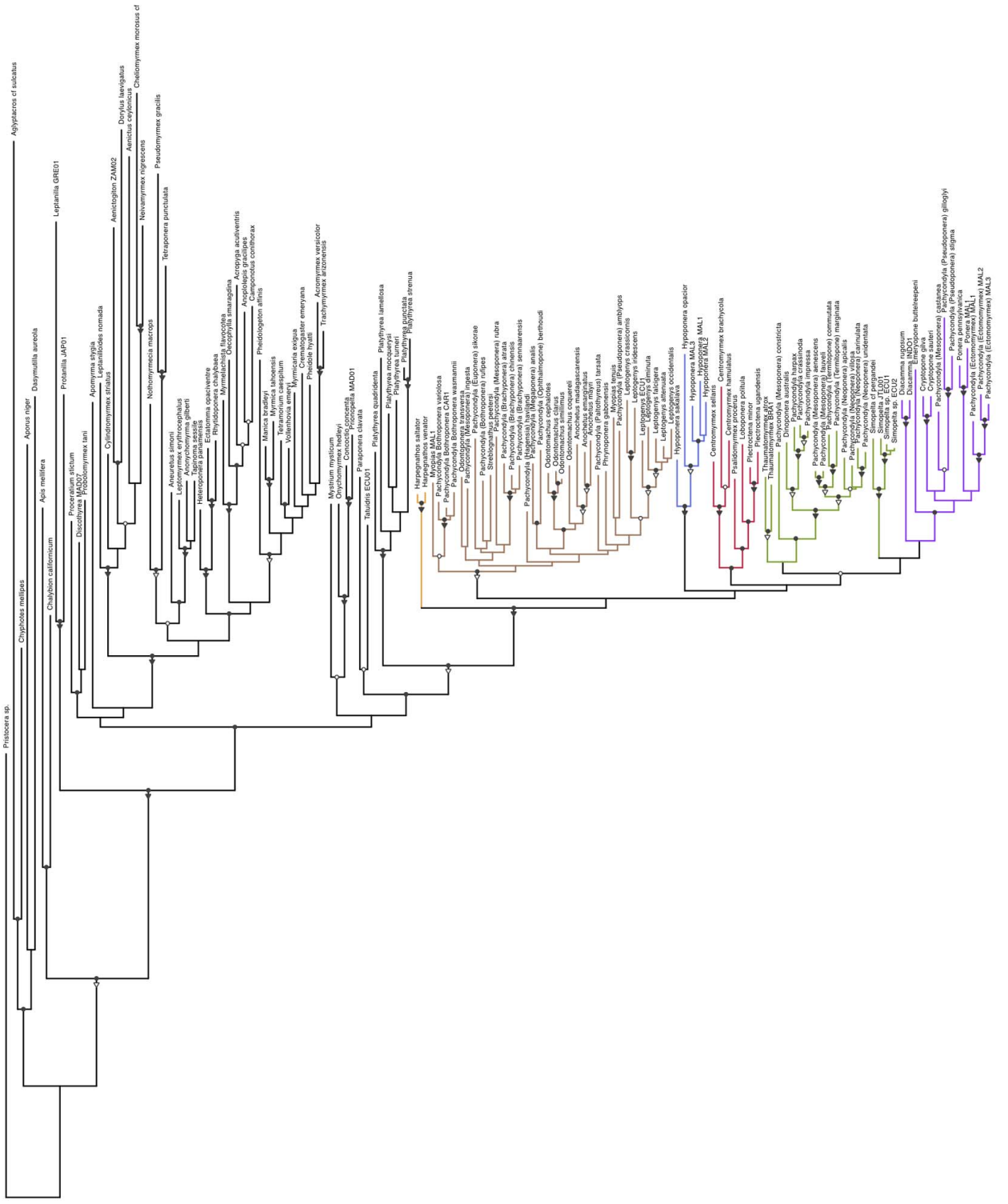
SUPPLEMENTAL FIGURE S1. Consensus phylogeny inferred by MrBayes analysis of the ALL_DATA matrix with all of CAD included for *Harpegnathos*, showing mean branch lengths. Genus groups within Ponerini are indicated by branch color. Clade support is indicated by solid (MrBayes BPP = 1.00) and empty (0.95 < BPP < 0.99) circles and solid (RAxML BS = 1.00) and empty (0.95 < BS < 0.99) triangles. This tree is available from TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13082).



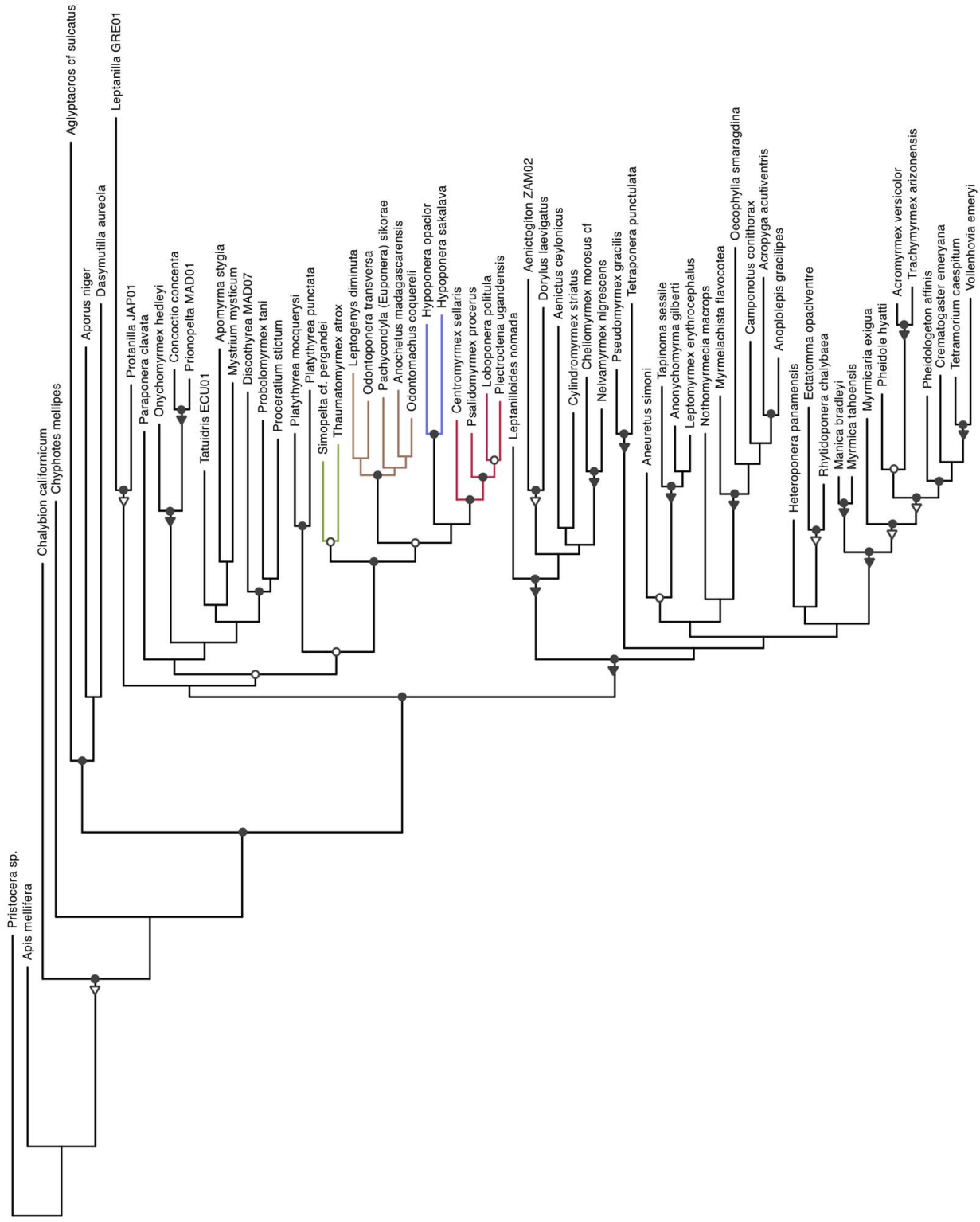
SUPPLEMENTAL FIGURE S2. Consensus phylogeny inferred by MrBayes analysis of the ALL_BUT_CAD matrix, showing mean branch lengths. Genus groups within Ponerini are indicated by branch color. Clade support is indicated by solid (MrBayes BPP = 1.00) and empty (0.95 < BPP < 0.99) circles and solid (RAxML BS = 1.00) and empty (0.95 < BS < 0.99) triangles.



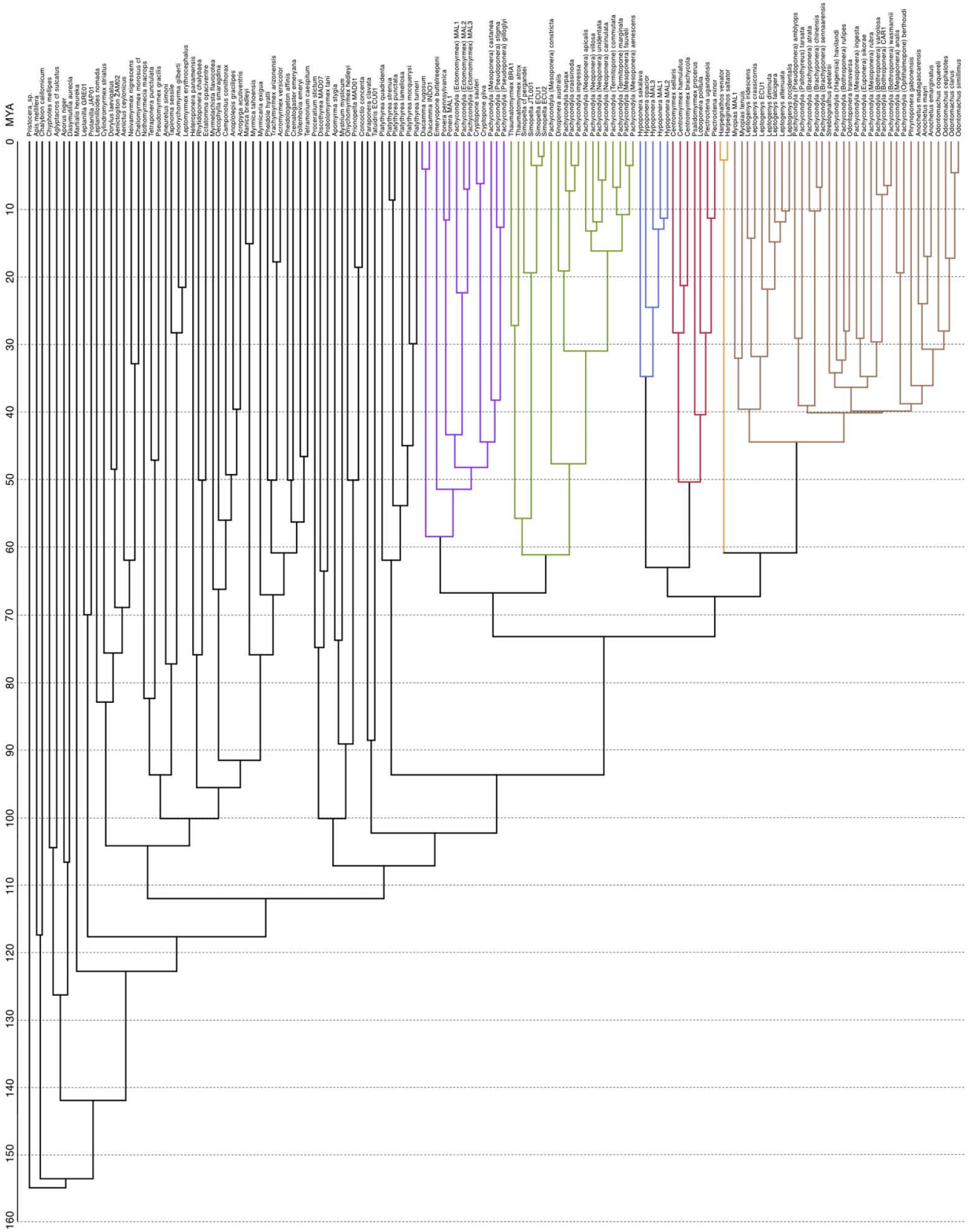
SUPPLEMENTAL FIGURE S3. Consensus phylogeny inferred by MrBayes analysis of the CAD matrix, showing mean branch lengths. Genus groups within Ponerini are indicated by branch color. Clade support is indicated by solid (MrBayes BPP = 1.00) and empty (0.95 < BPP < 0.99) circles and solid (RAxML BS = 1.00) and empty (0.95 < BS < 0.99) triangles.



SUPPLEMENTAL FIGURE S4. Consensus phylogeny inferred by MrBayes analysis of the WG_LWR_28S matrix, showing mean branch lengths. Genus groups within Ponerini are indicated by branch color. Clade support is indicated by solid (MrBayes BPP = 1.00) and empty (0.95 < BPP < 0.99) circles and solid (RAxML BS = 1.00) and empty (0.95 < BS < 0.99) triangles.



SUPPLEMENTAL FIGURE S5. Consensus phylogeny inferred by MrBayes analysis of the SECONDARY_GENES matrix, showing mean branch lengths. Genus groups within Ponerini are indicated by branch color. Clade support is indicated by solid (MrBayes BPP = 1.00) and empty (0.95 < BPP < 0.99) circles and solid (RAxML BS = 1.00) and empty (0.95 < BS < 0.99) triangles.



SUPPLEMENTAL FIGURE S6. Dated phylogeny (maximum clade credibility tree with mean node ages) inferred by BEAST analysis of the ALL_DATA matrix with a 155 Mya root age and all of CAD included for *Harpegnathos*. Genus groups within Ponerini are demarcated by branch color.