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TESTING THE MUSEUM VERSUS CRADLE TROPICAL BIOLOGICAL DIVERSITY HYPOTHESIS: PHYLOGENY, DIVERSIFICATION, AND ANCESTRAL BIOGEOGRAPHIC RANGE EVOLUTION OF THE ANTS

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Ants are one of the most ecologically and numerically dominant group of terrestrial organisms with most species diversity currently found in tropical climates. Several explanations for the disparity of biological diversity in the tropics compared to temperate regions have been proposed including that the tropics may act as a "museum" where older lineages persist through evolutionary time or as a "cradle" where new species continue to be generated. We infer the molecular phylogenetic relationships of 295 ant specimens including members of all 21 extant subfamilies to explore the evolutionary diversification and biogeography of the ants. By constraining the topology and age of the root node while using 45 fossils as minimum constraints, we converge on an age of 139–158 Mya for the modern ants. Further diversification analyses identified 10 periods with a significant change in the tempo of diversification of the ants, although these shifts did not appear to correspond to ancestral biogeographic range shifts. Likelihood-based historical biogeographic reconstructions suggest that the Neotropics were important in early ant diversification (e.g., Cretaceous). This finding coupled with the extremely high-current species diversity suggests that the Neotropics have acted as both a museum and cradle for ant diversity.

KEY WORDS: **Biogeography, divergence dating, Formicidae, molecular clock, Neotropics, phylogenetics.**

The discontinuity of species richness across the globe has long been of great interest to biogeographers, ecologists, and evolutionary biologists. In addition, biologists have observed some general patterns in species richness, such as the high species richness of the tropical rainforests (Wallace 1878; Fischer 1960; Pianka 1966; Janzen 1967), latitudinal gradients (the increase in species numbers from the poles to the tropics; Pianka 1966; Jablonski et al. 2006; Mittelbach et al. 2007), or the observation that, for some taxa (i.e., butterflies and angiosperms), the Neotropics are more diverse than the Paleotropics (Kier et al. 2005; Qian and Ricklefs 2008, and reference within). The origin and maintenance of tropical diversity has often been explained in terms of tropical forests being evolutionary "museums" or conversely "cradles" for biological diversity (Stebbins 1974; Stenseth 1984; Jablonski 1993; Gaston and Blackburn 1996; Chown and Gaston 2000; Jablonski et al. 2006; Wiens and Donoghue 2004), although relatively few studies have explored these hypotheses with data from terrestrial organisms. The concept of tropical rainforests acting as a museum for biological diversity predicts that taxa will be older in the tropics, have lower extinction rates, and have larger geographic range sizes (as is often found in the tropics) that positively correlate with their evolutionary persistence. However if

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the tropical rainforests are a cradle for biological diversity then we would expect to see high origination/speciation rates, new adaptive complexes arising within the area, and this region is acting as a center of origin for species diversity. A third "out of the tropics" (OTT) model has been proposed by Jablonski et al. (2006), in which taxa originate in the tropics and expand toward the poles without losing their tropical presence. No single variable or model alone—biotic or abiotic—has yet to explain the latitudinal species gradient and the higher species richness of the tropics, although empirical evidence supporting one or more of these hypotheses has been found. Traditionally, these patterns have been explained in term of environmental correlations (see Ricklefs 2004) or local species assemblages (i.e., community ecology). More recently, however, people have argued for the importance of historical biogeography in understanding global species patterns (see Weins and Donoghue 2004; Donoghue 2008).

Ants (Hymenoptera: Formicidae) are among the most ecologically dominant groups of animals on Earth: they engage in intricate symbioses with an array of other insects, plants, fungi, and bacteria, play a vital role in turning soil and scavenging in many ecosystems, prey on both plants and animals, function as serious agricultural pests while cultivating their own elaborate fungal gardens, and take part in a myriad of additional interactions that shape terrestrial ecosystems. Their ecological dominance has not been limited to the present, with fossil evidence suggesting that they have been involved in symbioses and ecological interactions for millions of years (Baroni Urbani 1980; Hölldobler and Wilson 1990; Grimaldi and Engel 2005; LaPolla 2005).

Over the past several years, substantial progress has been made in the understanding of the phylogenetic relationships of the major ant clades (Bolton 2003, 2012; Ohnishi et al. 2003; Astruc et al. 2004; Brady et al. 2006; Moreau et al. 2006; Rabeling et al. 2008; Moreau 2009). Although debate continues as to who the living sister lineage of the ants may be (Brady et al. 2006; Moreau et al. 2006; Rabeling et al. 2008; Kück et al. 2011), these same studies have provided much clarity to many other relationships among the ants. In addition, researchers are beginning to understand the factors that may have shaped ant diversification, such as the rise of the angiosperms (flowering plants) and their association with hemipterian insects (Moreau et al. 2006; Pie and Tscha 2009; Moreau and Bell 2011) and endosymbiotic bacteria (Russell et al. 2009).

Ants have an unusually rich fossil record with over 60 extant, modern genera represented, not to mention the large number of extinct lineages (over 650 fossil species in over 190 fossil genera; Perrichot 2012) with some fossil formations containing thousands of individual ant fossils (Dlussky and Rasnitsyn 2009). These fossils, distributed around the globe and spanning 100 million years, afford us the unique opportunity to investigate the timing of ant evolution by calibrating a molecular clock with minimum ages for many extant genera. With each new fossil unearthed, the timing of ant evolution has progressively changed (Grimaldi et al. 1997, 2002; Grimaldi and Agosti 2000; Nel et al. 2004; Engel and Grimaldi 2005; Wilson and Hölldobler 2005; Perrichot et al. 2007a,b). In particular, as older fossils have been discovered and assigned to the family Formicidae, the origin of the ants has been pushed back earlier in time. This is not a situation unique to ants (e.g., see Poinar and Danforth 2006; Bell et al. 2010), but it is best demonstrated in this group because they have left so many highly distinctive and identifiable amber and rock fossils. Sixteen of the 21 living subfamilies have fossil records that date back as early as the Late Cretaceous ~80 million years ago (Mya; Perrichot 2012), and the lack of fossil ants before the Albian, ~100 Mya, in the Cretaceous may reflect a bias in the fossil record, as Early Cretaceous fossil insect sites are relatively scarce (Perrichot et al. 2007a) and may not be due to a lack of presence in this time period. Our study brings together the parallel tracks of fossil discovery on the one hand and molecular clock analysis on the other as tools to understand the evolution and diversification of this ecologically important group.

With a rich and deep fossil record and previous molecular clock analyses that suggest that the extant crown-group ants may be 115–168 Mya (Brady et al. 2006; Moreau et al. 2006), this raises the question of how continental drift and historical biogeography may have shaped the current distribution of ant diversity. Although there are cases where the breakup of the continents may explain current distributions of ant lineages (army ants: Brady 2003; ectatommines: Lattke 2003), these cases are not common. Not surprisingly, the world's tropics harbor the highest diversity of ant species and biomass. A latitudinal gradient has been observed in ants with diversity decreasing from the equator toward the poles for both the number of genera (Dunn et al. 2010; McGlynn 2010; Guénard et al. 2012) and number of species (Kusnezov 1957; Jeanne 1979; Kaspari et al. 2004; Jenkins et al. 2011). If the rise of the angiosperm dominated forests were an important factor in the diversification of the ants (Moreau et al. 2006) then it would not be unexpected to find that ant evolution was tied to one or several of the tropical regions of the world. Also, if the historical biogeography of the ants is tied to the tropics, do shifts in their diversification correspond to major changes in their geographic distributions?

In this article we infer the phylogenetic relationships of the ants and explore the influence of a BEAST (Drummond and Rambaut 2007) "relaxed clock" dating method that allows fossil constraints to be treated as probability distributions as opposed to hard boundaries or fixed calibration points. We use the combined data from Moreau et al. (2006), Brady et al. (2006), and Rabeling et al. (2008), resulting in a matrix of 311 total taxa with 295 ingroup specimens including members of all 21 extant ant subfamilies and 45 minimum fossil calibrations across five nuclear

genes. We also explore the behavior of this method to assumptions, including constraining the root node versus not constraining the root node, simultaneously estimating the phylogeny and divergence dates, and the influence of our fossil priors. Finally, we use our time-calibrated trees (chronograms) to investigate the potential shifts in diversification rates across ants, as well as the role of historical biogeography on the evolutionary history of the ants.

Materials and Methods

MOLECULAR DATA

Molecular sequence data were collected from GenBank and TreeBASE, representing data from Moreau et al. (2006), Brady et al. (2006), and Rabeling et al. (2008). Only the five nuclear gene regions that were in common between the studies were included (*18S rDNA*, *28S rDNA*, *abdominal-A*, *long-wavelength rhodopsin*, and *wingless*). The aligned matrices of Moreau et al. (2006) and Brady et al. (2006) were downloaded and aligned to one other in Mesquite (Maddison and Maddison 2011). As in Moreau et al. (2006) and Brady et al. (2006), ambiguously aligned sites were excluded from phylogenetic analyses. The genes aligned relatively unambiguously and the alignment of the protein coding genes was confirmed by translation to amino acids. Missing data were all coded as "?." The single taxon addition of Rabeling et al. (2008) was then aligned to this larger matrix. Newly aligned data matrices for the 311 taxa and 3324 basepairs were deposited into TreeBASE (study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S11283; www.treebase.org).

PHYLOGENETIC INFERENCE

Maximum parsimony criterion searches were performed using PAUP* (Swofford 2002). Parsimony searches were conducted on the complete concatenated dataset using the random stepwise addition option of the heuristic search for 500 replicates with tree bisection–reconnection (TBR) branch swapping, collapse of zero-length branches, and equal weighting of all characters. If searches produced more than one tree, a strict consensus was performed to summarize data analyses. To measure the robustness of branching patterns of the parsimony trees, bootstrap analyses (bs; Felsenstein 1985; Hillis and Bull 1993) were executed for 500 replicates.

Maximum likelihood (ML) methods were also used to search for tree topologies. For each dataset, PORN* (Bell 2001) was used to determine the appropriate evolutionary model for each partition based on likelihood values calculated with PAUP*. The Akaike information criterion (AIC) was used to evaluate the fit of competing models. In all case the GTR + Γ model was selected as the most appropriate.

Likelihood searches were performed using the software RAxML version 2.0 (Stamatakis et al. 2005) with a single model of molecular evolution underlying the complete concatenated matrix and a partitioned analysis allowing for estimating of model parameters for individual genes. Each partition was given its own GTRGAMMA model of sequence evolution. ML branch support was evaluated using 300 bs replicates (Felsenstein 1985). Search parameters for bs tests were identical to those of individual likelihood searches.

In addition to maximum parsimony and ML, tree topologies were also inferred using Bayesian methods using Markov chain Monte Carlo (MCMC) methods to sample the posterior distribution of trees. We used two approaches in our Bayesian analyses: (1) we assumed a single common model across all molecular data (one partition) and (2) a separate model for each gene (five partitions). In each case the underlying model was a GTR + Γ model of sequence evolution (based on AIC). For parameters across partitions we unlinked the substitution rates, character state frequencies, and γ-shaped parameter alpha (α). All other parameters (i.e., priors) were left at their default values. Posterior probabilities were calculated using the resulting trees after burn-in. Bayesian analyses were conducted with the MPI-enabled version of MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), splitting runs and chains across processors. Each analysis consisted of six independent runs, with four chains (one cold and three hot) for 36 million generations. Chains were sampled every 100 generations, and burn-in was determined based on visual inspection of log-likelihood over time plots using Tracer version 1.3 (http///evolve.zoo.ox.ac.uk/software.html?id=tracer). Convergence of runs was ensured by only accepting analyses where the average standard deviation of split frequencies was below 0.01.

To test possible alternative rootings and the potential effect of long-branch attraction (LBA), the likelihood-based Shimodaira–Hasegawa test (Shimodaira and Hasegawa 1999) was implemented in PAUP* with 10,000,000 RELL bs pseudoreplicates. We compared our ML topology to five alternative rooting scenarios that varied in where the position of the outgroups was placed: (1) Martialinae as sister to all remaining ants; (2) Leptanillinae + Martialinae as sister to all remaining ants; (3) Amblyoponinae as sister to all remaining ants; (4) Poneroid clade (Amblyoponinae + Ponerinae + Agroecomyrmecinae + Paraponerinae + Proceratiinae) as sister to all remaining ants; and (5) Leptanillinae + Martialinae + Poneroids as sister to all remaining ants.

DIVERGENCE TIME ESTIMATION

Divergence dating for most recent common ancestors (MRCA) was estimated using the software package BEAST version 1.7.4 (Drummond and Rambaut 2007). Fossil data were taken from both Moreau et al. (2006) and Brady et al. (2006). Fossils used as minimum calibration points are listed in Table S1. The model of molecular evolution was set to GTR + G with the molecular

clock model set as an uncorrelated log-normal relaxed clock with a Yule process, speciation tree prior. For the fossil calibration, a lognormal prior distribution was implemented with an offset corresponding to the minimum fossil age, log(mean) of 1.0, and log(SD) of 1.0. Several short initial runs were preformed in BEAST to define the parameter-specific settings for the MCMC search for each dataset. The MCMC length was 50,000,000 with parameters sampled every 1000 steps. A burn-in of 10% was implemented in TreeAnnotator after analysis in Tracer and the tree and credible intervals were visualized in FigTree version 1.3.1 (Rambaut 2009). Five separate divergence dating analyses were implemented using the 45 fossils listed in Table S1 assigned as minimum ages to the root of the clade + sister lineage to which they belong. These five analyses were implemented to account for the effects of constraining the age of the root node, the inclusion of multiple individuals per species, and constraining the topology during analysis since recent the findings of Heled and Drummond (2012) suggest that with large numbers of calibration points searching for the topology may not be appropriate. The five divergence dating analyses we implemented are as follows: (1) ML topology excluding duplicate species enforced and root node constrained to a maximum age of 185 Mya (following Brady et al. 2006); (2) ML topology enforced excluding duplicate species and root node unconstrained (no max age for the root node); (3) ML topology enforced including all taxa and root node constrained to a maximum age of 185 Mya; (4) ML topology enforced including all taxa and root node unconstrained (no max age for the root node); and (5) topology estimated during analysis including all taxa and root node constrained to a maximum age of 185 Mya. Adequate mixing for all BEAST searches was assessed by only accepting searches that achieved high effective sample size values for all parameters. In addition, posterior probabilities of each node were compared across independent runs to further ensure convergence. Finally, we ran all five BEAST analyses without the aligned sequence data to explore the influence of our priors (Drummond et al. 2006). This allows researchers to investigate the influence of the prior distribution and its contribution to the posterior.

SHIFTS IN DIVERSIFICATION RATES

To explore patterns of diversification across the ant phylogeny, we employed several diversification statistics. To test the null hypothesis of constant diversification rates through time, we used both the birth–death (BD) likelihood and pure-birth (PB) likelihood methods of Rabosky (2006) and compared them to three models that relax the assumption of rate constancy in diversification. Under these models, the method provides the most likely break points for models with two or more rates. We used the Akaike information criterion (AIC) to evaluate the five different diversification models. We selected the best-fitting model by comparing the difference in AIC values (ΔAIC) between the best rate-constant model and the AIC values of the rate-variable models. Type I error was evaluated by creating a null distribution of expected differences in AIC values for the best-fit rate-constant (AIC_{RC}) and rate-variable (AIC_{RV}) models. We simulated 10,000 PB trees with taxon sampling in Phylogen version 1.1 (Rambaut 2002).

Lineage-through-time (LTT) plots were then constructed using the R package APE (Paradis et al. 2004). We plot our estimated dated tree, the ages inferred with molecular data excluded, and the 95% range of branching times across the 10,000 simulated PB trees. All analyses assumed an extant species diversity of at least 12,199 species for the ants (i.e., ingroup taxa).

To identify potential diversification rate shifts within ants, we applied the modeling evolutionary diversification using stepwise Akaike information criterion (MEDUSA) method (Alfaro et al. 2009; Slater et al. 2010) to our BEAST chronogram for ants with duplicate species and outgroups excluded. Along with our tree, we used current taxonomic data (i.e., number of valid species per genus; Bolton 2012) to construct a diversity tree. As defined by Alfaro et al. (2009), a diversity tree is a time-calibrated phylogenetic tree (or chronogram) where each tip has been given a species richness value, based on current taxonomic diversity. The MEDUSA method used here is a stepwise algorithmic procedure that (1) fits a BD model to the diversity tree using both, a phylogenetic, as well as taxonomic likelihood function developed by Rabosky et al. (2007); (2) the AIC score of this two-parameter model (a single birth rate and an extinction rate) is compared with a five-parameter model where the birth and death rates are allowed to shift on the optimal branch of the phylogeny (Slater et al. 2010). If this five-parameter model (two birth rates, two death rates and a shift-location parameter) produces a substantial improvement in the AIC score, the five-parameter model is stored and compared with the best eight-parameter model. This stepwise procedure continues until there are no significant improvements in the AIC values, by adding more parameters. For this study, we set the threshold at four units, or greater, for an improvement in AIC values (Burnham and Anderson 2002). In this framework, by allowing 20 net diversification rates within a single phylogeny, there are a total of 39 parameters (20 rate values, plus 19 inferred rate shifts).

BIOGEOGRAPHIC RANGE EVOLUTION

To infer the ancestral biogeographic ranges across the ant phylogeny, we implemented the likelihood-based program package Lagrange version 2 (Ree et al. 2005; Ree and Smith 2008) with duplicate species and outgroups excluded from our inferred ML topology. Lagrange implements a dispersal–extinction–cladogenesis model of range evolution, which incorporates branch length information and permits dispersal rates between

biogeographic areas to change through time. All genera included were assigned to one or more of six broad biogeographic regions corresponding to World Wildlife Fund terrestrial ecozones: (1) Palearctic (Eurasia and North Africa); (2) Nearctic; (3) Afrotropic; (4) Neotropic; (5) Australasia (Australia, New Guinea, and neighboring islands—northern boundary of Wallace's line); (6) Indo-Malaya/Oceania (southeast Asia and Pacific oceanic islands) following extant ant genera distribution data of Guénard et al. (2010) using only categories "present" and "likely present" to score the genus as present in one or more of the above biogeographic locations. Three separate ancestral biogeographic range analyses were conducted with identical assigned genera current ranges: (1) equal movement between all regions with equal dispersal constraints allowed with no information included regarding time (unconstrained); (2) dispersal between ranges were constrained (adjacency matrix) according to current proximity of regions with no information included regarding time (dispersal only); and (3) dispersal between ranges were constrained, although no dispersals were completely prohibited (0.1 = unlikely; 0.5 = possible; 1.0 = likely) based on continent distributions/proximity across four broad geologic time periods (Scotese 2010) as follows: present–50 Mya, 50–94 Mya, 94–150 Mya, and 150–200 Mya (although the root node of the tree only dated to ∼170 Mya; dispersal and time). For this last biogeographical range evolution analysis (dispersal and time), we assumed that dispersal to distant biogeographic regions could change through time but we never completely prohibited movement across geographic defined regions. For example, dispersal between the Afrotropical and Nearctic regions decreased through time to the present as the continents drifted apart and following the time intervals outlined above we set dispersal symmetric between these regions as very likely (1.0) between 200 and 150 Mya, possible (0.5) for 150–94 Mya, and unlikely although still possible (0.1) for 94 Mya–present.

Results

To infer the evolutionary history of the ants, we combined the molecular datasets of Moreau et al. (2006), Brady et al. (2006), and Rabeling et al. (2008). Our concatenated dataset include members of all 21 extant ant subfamilies, 311 total taxa with 295 ingroup ant specimens, and 45 minimum fossil calibrations across five nuclear genes. From these data we were able to infer the phylogenetic relationships, investigate the timing of diversification for the major lineages, and infer the role biogeography played in the evolutionary history of the ants.

PHYLOGENETIC INFERENCE

The phylogenetic relationships recovered in this analysis are largely congruent with the topologies recovered in the Moreau et al. (2006), Brady et al. (2006), and Rabeling et al. (2008) analyses (Fig. 1). In all analyses, the ant family, Formicidae, is recovered as monophyletic with high support. Representatives from all 21 subfamilies were included and only the Cerapachyinae were not recovered as a monophyletic group, as has been found in previous studies (Brady 2003; Brady and Ward 2005; Brady et al. 2006; Moreau et al. 2006). Also, as found in previous molecular phylogenetic studies, the leptanillines were recovered as sister to all extant ants (Saux et al. 2004; Brady et al. 2006; Moreau et al. 2006; Kück et al. 2011), which are further split into two clades: Poneroid and Formicoid. Unfortunately, due to weak support in the basal portion of the phylogeny, we cannot say with great certainty whether the Leptanillinae or Martialinae are the true sister lineage, although in our analyses the Leptanillinae were consistently recovered topologically as sister to all remaining extant ants (also see alternate rooting analyses below).

Parsimony, ML (concatenated and partitioned), and Bayesian inference (concatenated and partitioned) all resulted in similar topologies. Where topologies differed there was low support values across methods for those relationships. For example, only the Bayesian partitioned analysis recovered *Cerapachys augustae* and *Sphinctomyrmex* spp. + *Cerapachys larvatus* as sister to the army ant subfamilies Aenictinae and Ecitoninae, with low posterior probability (0.76). All other analyses collapsed this relationship to a polytomy within the larger dorylomorph clade. The relationships and support recovered across the four analyses (Bayesian partitioned, ML partitioned, ML concatenated, and maximum parsimony concatenated) are presented in Figure S1.

The results of our tests of alternate rooting and LBA analyses using the likelihood-based Shimodaira–Hasegawa test with 10,000,000 RELL bs pseudoreplicates resulted in the following: ML topology with Leptanillinae as sister to all other extant ants (best tree): $-\ln L = 104486.15108$; (1) Martialinae as sister to all remaining ants (not significantly different): $-\ln L = 104486.22526$, difference $-\ln L = 0.07419$, P-value 0.7637878; (2) Leptanillinae + Martialinae as sister to all remaining ants (not significantly different): $-\ln L = 104486.22526$, difference $-\ln L = 0.07419$, P-value 0.7637436; (3) Amblyoponinae as sister to all remaining ants (significantly different): $-\ln L = 104530.65869$, difference $-\ln L = 44.50761$, P-value < 0.0015073; (4) Poneroid clade (Amblyoponinae + Ponerinae + Agroecomyrmecinae + Paraponerinae + Proceratiinae) as sister to all remaining ants (significantly different): $-\ln L = 104510.35531$, difference $-\ln L = 24.20424$, P-value < 0.0149476; and (5) Leptanillinae + Martialinae + Poneroids as sister to all remaining ants (significantly different): $-\ln L = 104510.35531$, difference $-\ln L = 24.20424$, P-value < 0.0149480. These results support the sister relationship of Leptanillinae, Martialinae, or (Leptanillinae + Martialinae) to the remaining ants and do not support other alternate rooting scenarios as found by Brady et al. (2006).

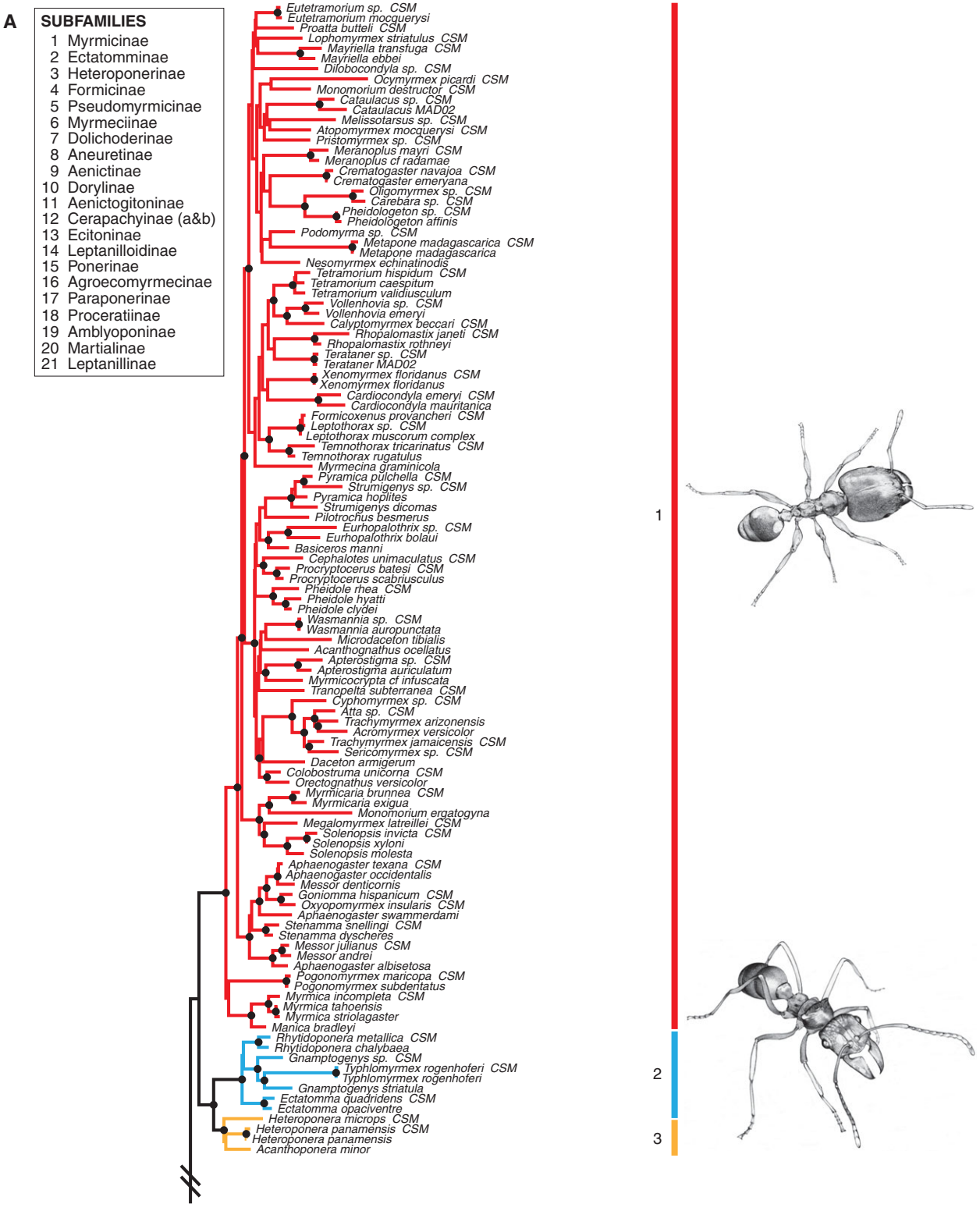

Figure 1. Phylogeny of the ants estimated under maximum likelihood for the partitioned analysis (five partitions) with branch lengths proportional to change with Bayesian posterior probabilities above 95% denoted as solid dots on nodes. Subfamily affiliation denoted using colors, numbers, and legend on figure. Species labels followed by "CSM" are from Moreau et al. (2006) and all other taxa are from Brady et al. (2006) except *Martialis heureka*, which is from Rabeling et al. (2008). Illustrations by Alexandra C. Westrich in order from top to bottom: *Pheidole*, *Ectatomma*, *Polyrhachis*, *Linepithema*, *Eciton*, *Odontomachus*, *Amblyopone*, and *Leptanilla*. *Pheidole, Linepithema,* and *Amblyopone* illustrations modified from photographs ©Alexander Wild and used with permission. (A) Top portion of the tree; (B) middle portion of the tree; and (C) the bottom portion of the tree.

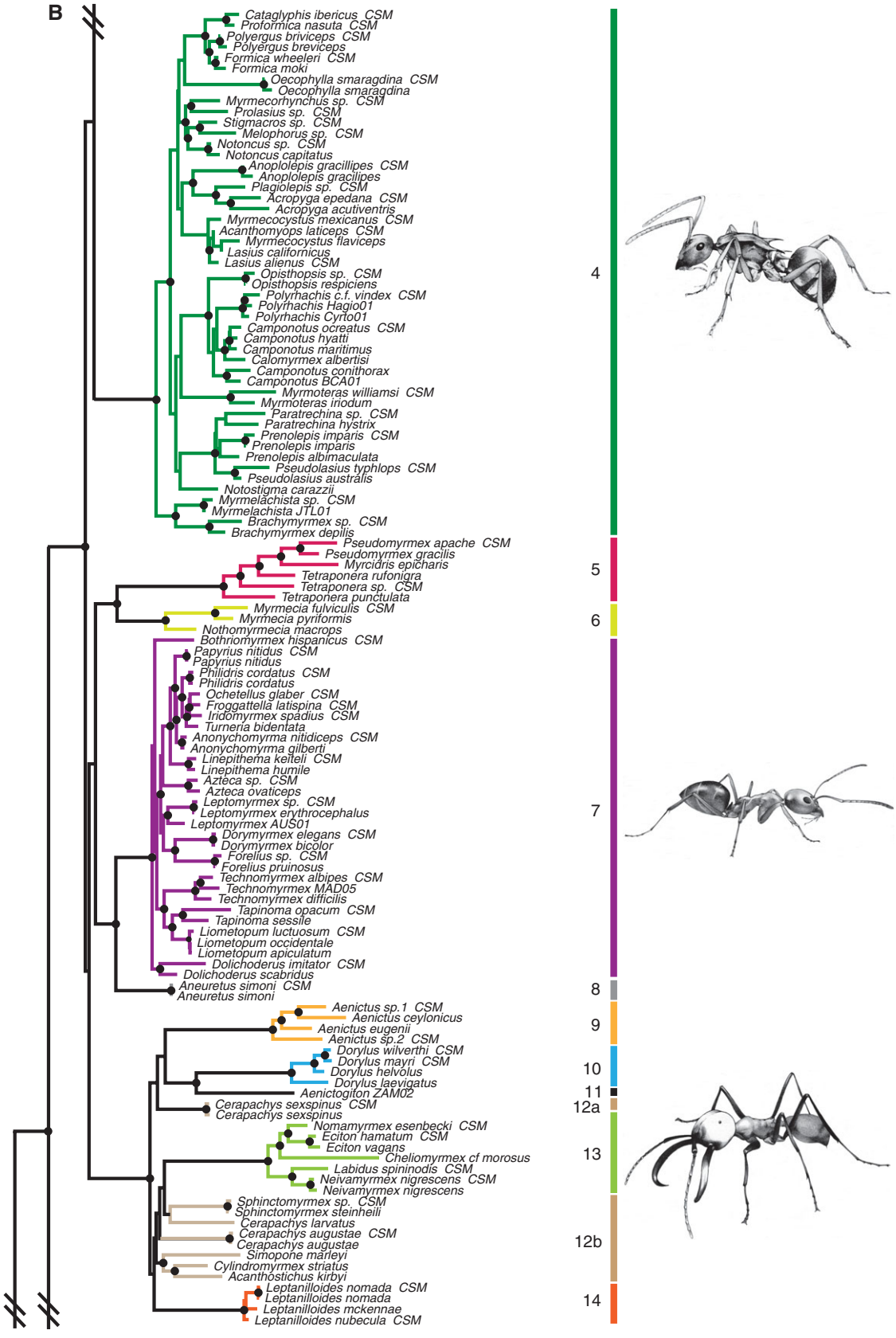

Figure 1. Continued.

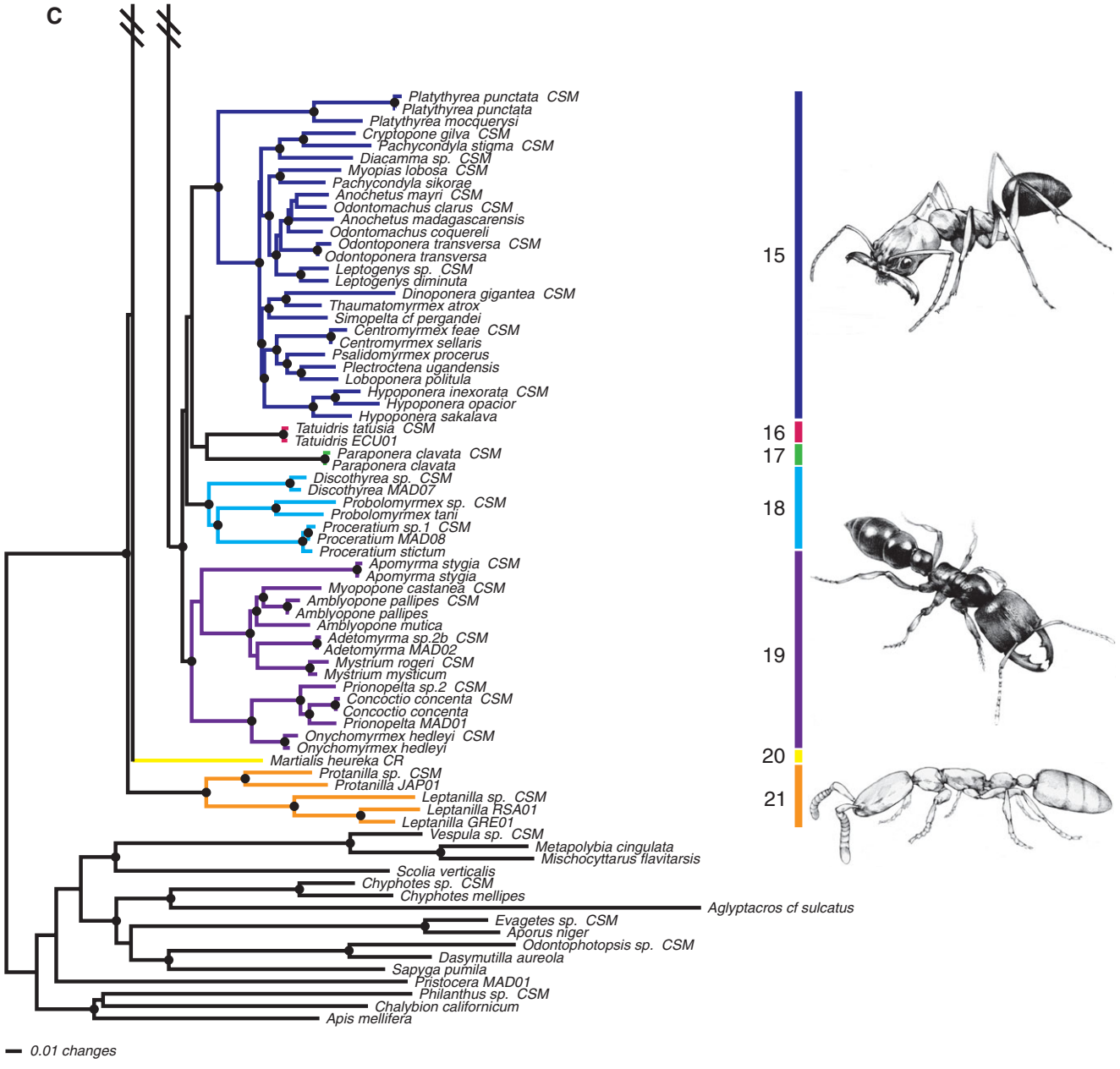

Figure 1. Continued.

Interestingly our analyses recovered (Myrmicinae [Ectatomminae + Heteroponerinae]) as a clade, although this relationship is not statistically supported. The Ectatomminae and Heteroponerinae were long considered members of the "poneromorph" clade (Bolton 2003) based on overall morphology until molecular evidence demonstrated their affinities within the formicoid clade (Moreau et al. 2006), although Brown (1958) suggested this close relationship between the Myrmicinae and Ectatomminae (which contained Heteroponerinae at the time) based on several morphological characters from males (mesosoma, wings, and genitalia) and all castes (integumental consistency, proventricular structure, propodeal form, and armament). Although our phylogeny lacks support within many of the major clades, several detailed recent studies have provided clarity into the phylogenetic relationships above the species level including army ants (Brady 2003), Formicinae (Johnson et al. 2003), Myrmeciinae (Ward and Brady 2003), Amblyoponinae (Saux et al. 2004), Pseudomyrmecinae (Ward and Downie 2005), poneroid subfamilies (Ouellette et al. 2006), fungus-growing ants (Schultz and Brady 2008), *Prenolepis* genus-group (LaPolla et al. 2010), and Dolichoderinae (Ward et al. 2010).

Several genera were not recovered as monophyletic in our analyses. In most cases their non-monophyly has been suggested in previous molecular phylogenetic studies and was also

found in our study: *Amblyopone* (Saux et al. 2004; this study); *Aphaenogaster* (Brady et al. 2006; Moreau 2008; this study); *Camponotus* (Brady et al. 2006; this study), *Cerapachys* (Brady 2003; Brady and Ward 2005; Brady et al. 2006; Moreau et al. 2006; this study), *Messor* (Brady et al. 2006; Moreau 2008; this study); *Monomorium* (this study); *Pachycondyla* (Schmidt 2009; this study); *Pyramica* (Baroni Urbani and De Andrade 2007; this study); *Strumigenys* (Baroni Urbani and De Andrade 2007; this study); and *Tetraponera* (Ward and Downie 2005; Brady et al. 2006; this study).

Although the ants (Formicidae) are consistently recovered as a monophyletic group, the sister lineage to the ants still remains unclear. Unfortunately neither previous (Brady et al. 2006; Moreau et al. 2006; Pilgrim et al. 2008) nor our current analyses provides clear insights into this potentially challenging problem.

DIVERGENCE TIME ESTIMATION

For each of the BEAST analyses we exclude 10% of the MCMC as our burn-in. From the post–burn-in sample of trees, we recovered an age ranging from 139.4 to 157.7 Mya for the age of crown-group ants across the five analyses implemented in BEAST (Drummond and Rambaut 2007; Table 1). For the molecular clock analysis with the ML topology constrained, duplicate species excluded and the root node constrained to a maximum of 185 Mya, we recovered an age of 148.0 (highest posterior density [HPD]: 127.6–168.8) Mya for the crown-group ants. In the second analysis with the ML topology constrained, duplicate species excluded and the root node not constrained, we recovered an age of 157.7 (HPD: 129.3–191.7) Mya for the crown-group ants. When the ML topology was enforced with all taxa included during the analysis and the root node constrained to a maximum of 185 Mya, we recovered an age of 140.7 (HPD: 118.5–164.0) Mya for the crown-group ants. Again enforcing the ML topology with all taxa included, but not constraining the root node resulted in an age of 148.3 (HPD: 120.2–175.9) Mya for the crown-group ants. Finally, when estimating the topology and divergence dates simultaneously while constraining the root node to a maximum of 185 Mya resulted in an age for the crown-group ants of 139.4 (HPD: 119.8–161.3) Mya. During this last analysis, the topology inferred during the divergence time estimation in BEAST largely agreed with the relationships recovered in the previous phylogenetic analyses (Fig. 2) and in most cases the areas of conflict were not well-supported across any analysis (Figs. 1, S1). It should be noted that this analysis also recovered the Leptanillinae as sister to all remaining ants with Martialinae then sister to the poneroid and formicoid clades.

As found for Moreau et al. (2006) and Brady et al. (2006), the fixed calibration point (in both of these cases the root node was given a maximum age) resulted in the penalized likelihood (PL) method pushing the root node age up against this maximum value. Evaluating methods suggests that PL can be highly influenced by the maximum age of the root node or fixed calibration point compared to our findings in the Bayesian framework of BEAST. Although we found across our analyses that the 95% HPD consistently contained the upper bound or maximum age of the root node (185 Mya) when constrained for all ingroup and outgroup taxa (root node age of ingroup and outgroup HPD for ML topology constrained = 170.0–185.1 Mya; root node age of ingroup and outgroup HPD for BEAST estimated topology = 165.8–185.0 Mya), the ages for the major ant lineages were not very different when the root node was not constrained (root node age of ingroup and outgroup HPD when ML topology constrained and no maximum age for root node = 178.2–265.7 Mya). Regardless of whether we enforced a maximum age on the root node or not, we recover similar ages for the crown-group ants across most analyses (Table 1).

With the advent of Bayesian methods for phylogenetic inference, systematists have become aware that the posterior distribution is a product of both the prior distribution (imposed by the investigator) and the likelihood function (information coming from your data/observations). Strong priors can override any signal from the data, just as vague, or uninformative, priors may get swamped by the signal in the data. Results from sampling of the prior distribution suggest that our ages are being driven in large part by the priors on our fossil calibrations, although we infer older ages for most of the major lineages when the molecular data are excluded. This influence of our fossil priors may also explain why we recover such similar ages regardless of whether we constrain the maximum age of the root node (Table 1; Fig. 3).

SHIFTS IN DIVERSIFICATION RATES

We found that our PB diversification model produced a low average rate of diversification for ants (birth rate = 0.021 lineages per Mya, ln $L = -67.48$), which poorly explains the diversity of most of the extant clades of ants. That is, most of the extant lineages of ants have either too many or too few species, given their estimated age, to be an outcome of this PB rate. The BD model estimated the same speciation rate with an extinction fraction of zero, and thus was not a better fit for our data. In comparing rate variable models, we found that the "yule2rate" model was selected as the best fitting model (rate 1 = 0.042 lineages per Mya, rate 2 = 0.0163 lineages per Mya, ln $L = -42.58$, shift point = 56.7). The observed ΔAIC (45.8) was considered significant ($P = 0.0037$) when compared to a null distribution of ΔAIC values calculated for simulated trees of equal size and missing taxa to that of our empirical data. A recent study by Rabosky (2010) suggests that these simple models may fit data poorly, especially when there may be no relationship between clade age and their species richness. In our analyses using the MEDUSA method that accounts for current species diversity for each terminal, we identified

Table 1. Crown-group age estimates for major ant lineages across studies and methods. PL = penalized likelihood; BEAST = Bayesian relaxed clock; HPD = highest posterior density. Ages in millions of years ago (Mya).

	Moreau et al. (2006): PL min (root node constrained to 200 Mya)	Moreau et al. (2006): PL max (root node constrained to 250 Mya)	Brady et al. (2006): PL min (root node constrained to 145 Mya)	Brady et al. (2006): PL max (root node constrained to 185 Mya)	Moreau and Bell (this study): BEAST (No duplicate species ML topology enforced and root node constrained to max 185 Mya [95% HPD])	Moreau and Bell (this study): BEAST (No duplicate species ML topology enforced and root node not constrained [95% HPD])	Moreau and Bell (this study): BEAST (Multiple species ML topology enforced and root node constrained to max 185 Mya [95% HPD])	Moreau and Bell (this study): BEAST (Multiple species ML topology enforced and root node not constrained [95% HPD])	Moreau and Bell (this study): BEAST (Multiple species topology estimated and root node constrained to max 185 Mya [95% HPD])	Moreau and Bell (this study): BEAST (Molecular data excluded, ML topology enforced and root node constrained to max 185 Mya [95% HPD])
Myrmicinae	99.8 ± 4.2	114.0 ± 4.5	82 ± 4.3	89 ± 5.8	82.0	81.1	78.8	80.1	78.3	89.8
					(73.7–89.9)	(72.6–89.4)	(71.0–86.3)	(71.4–88.0)	(68.1–86.6)	(79.5–99.3)
Formicinae	92.0 ± 0.2	101.4 ± 3.8	77 ± 3.5	82 ± 4.4	79.9	79.7	75.0	74.9	79.2	90.3
					(68.5–89.5)	(69.3–88.6)	(64.8–85.8)	(64.9–83.1)	(69.4–90.1)	(76.8–101.8)
Dolichoderinae	85.6 ± 2.2	96.6 ± 1.9	71 ± 3.9	75 ± 5.1	65.3	66.3	58.3	53.2	58.6	66.0
					(58.8–71.9)	(59.8–73.3)	(54.5–61.8)	(55.4–61.6)	(54.3–61.7)	(56.4–78.6)
Ponerinae	110.7 ± 6.3	131.5 ± 5.9	79 ± 6.3	90 ± 8.1	59.6	58.9	56.4	55.9	57.6	59.6
					(54.2–65.7)	(53.4–70.7)	(52.6–60.0)	(52.3–59.6)	(52.9–60.4)	(53.2–65.0)
Leptanillinae	102.4 ± 4.1	123.0 ± 3.4	74 ± 8.3	86 ± 10.2	94.2	103.7	88.1	87.1	79.7	72.1
					(67.6–126.9)	(66.9–146.3)	(56.8–118.6)	(49.9–121.8)	(43.0–107.2)	(13.8–136.1)
Dorylomorphs	99.2 ± 3.4	116.9 ± 4.1	77 ± 4.9	88 ± 5.9	81.5	80.6	77.8	82.0	77.9	94.6
					(68.1–93.2)	(63.8–95.9)	(62.2–92.6)	(62.1–100.8)	(62.7–90.7)	(64.6–119.1)
Formicoid clade	124.7 ± 6.5	147.0 ± 8.2	105 ± 3.5	119 ± 5.5	110.6	109.6	107.0	107.1	107.8	119.6
					(104.2–118.2)	(102.8–119.2)	(100.0–114.5)	(100.0–115.0)	(100.0–115.9)	(103.0–139.4)
Poneroid clade	128.2 ± 5.9	152.4 ± 6.2	100 ± 6.1	115 ± 8.2	87.7	86.7	85.0	80.1	82.1	103.7
					(69.1–107.4)	(67.7–111.1)	(66.3–106.9)	(65.9–102.5)	(64.3–103.3)	(70.8–135.4)
Formicidae	**140.6 ± 8.0**	**168.8 ± 7.6**	**116 ± 3.8**	**133 ± 6.0**	**148.0**	**157.7**	**140.7**	**148.3**	**139.4**	**153.3**
					(127.6–168.8)	**(129.3–191.7)**	**(118.5–164.0)**	**(120.2–175.9)**	**(119.8–161.3)**	**(127.4–179.9)**

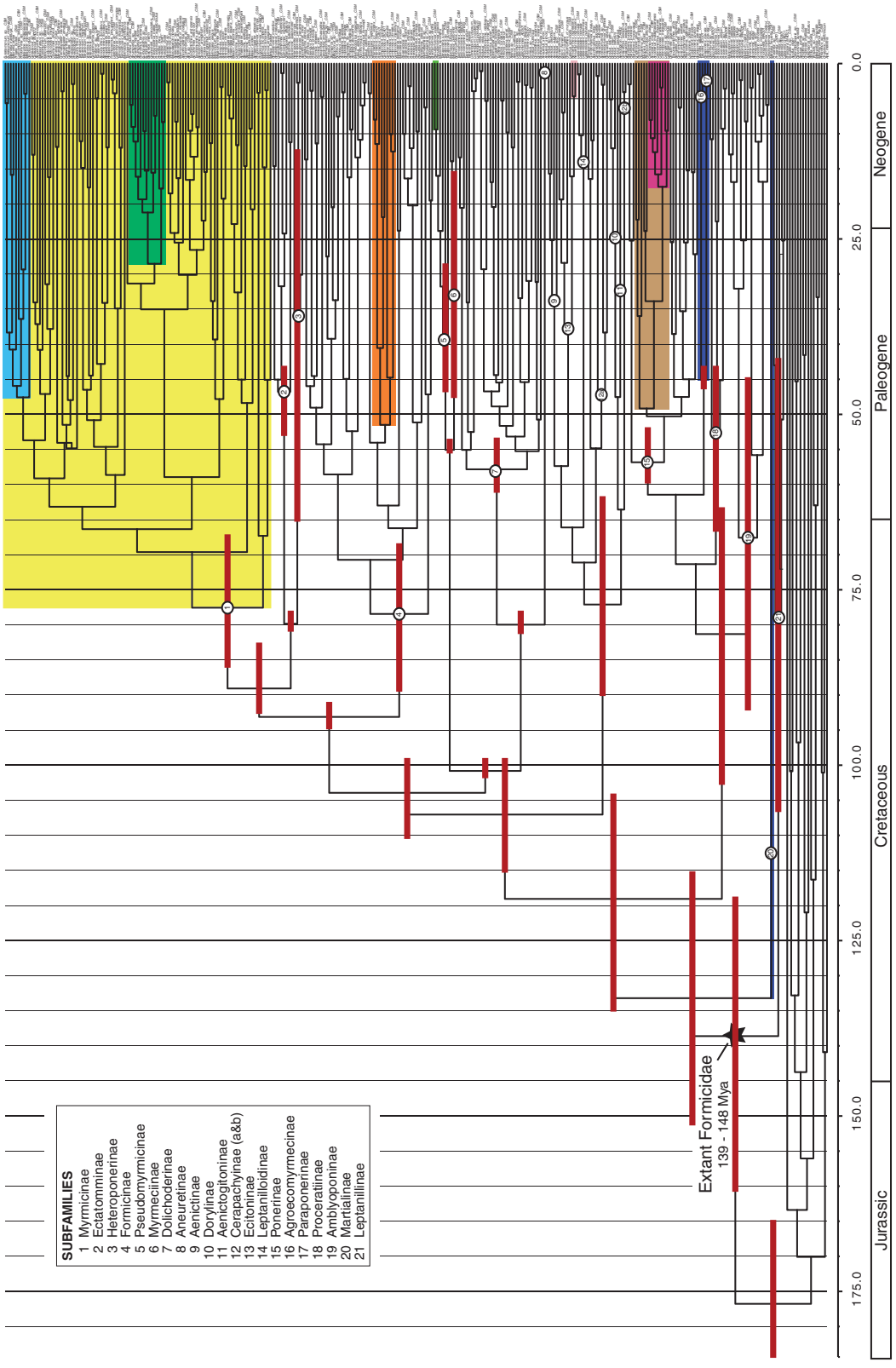

Figure 2. Dated chronogram of the ant phylogeny with outgroups included. The topology and divergence dates were inferred using BEAST with a maximum age of 185 Mya for the root node. The red bars indicate 95% highest posterior density (HPD) for major nodes within the crown-group Formicidae. Subfamilies are denoted using numbers and legend on figure following Figure 1. Scale bar is in millions of years before the present. Colored boxes overlaid on clades denote the 10 periods in which the MESUDA method detected a significant change in the tempo, or rate, of diversification across the ant phylogeny as outlined in Table 2 (clade colors defined in Table 2; four dark blue boxes denote clades with significant rate decreases, all others indicate rate increases).

Table 2. Results from modeling evolutionary diversification using stepwise Akaike information criterion (MEDUSA) diversification analysis. Clades with unusual diversification rates (box colors correspond to Fig. 2). (+) indicate a significant rate increase. (−) indicate a significant rate decrease. r = net diversification rate ($\lambda - \mu$, where λ = speciation rate, and μ = extinction rate), and ε = relative extinction rate ($\varepsilon = \mu/\lambda$). Crown-group (CG) age is denoted in millions of years. Number of species refers to extant species within the clade denoted. Background rate was determined for the number of extant species represented by genera included in this study (not total number of species worldwide).

Clade	r	ε	CGAge	No. of species
(+) Myrmicinae (yellow box)	0.1015903	1.064077E−06	82	5647
(+) *Dilobocondyla*, *Eutetramorium*, *Lophomyrmex*, *Mayriella*, *Proatta* (light pink box nested in yellow box)	0.04408837	1.560960E−06	59	33
(+) *Basiceros*, *Cephalotes*, *Eurhopalothrix*, *Pheidole*, *Pilotrochus*, *Procryptocerus*, *Pyramica*, *Strumigenys* (green box nested in yellow box)	0.1382280	0.7008019	38	2010
(+) Formicinae (light blue box)	0.04178411	0.97279721	80	2624
(+) Dolichoderinae (pink box)	0.07603380	9.813846E−07	65	655
(+) *Anochetus*, *Leptogenys*, *Odontomachus*, *Odontoponera* (orange box)	0.0587749	0.9860879	21	419
(−) *Formicoxenus*, *Leptothorax*, *Temnothorax* (dark blue box nested in yellow box)	4.447396E−03	0.999177859	39	361
(−) *Aneuretus* (dark blue box)	1.528812E−16	7.941261E−03	82	1
(−) *Paraponera*, *Tatuidris* (dark blue box)	8.865090E−03	1.033897E−07	47	2
(−) Leptanillinae + Martialinae (dark blue box)	0.01588045	0.77983549	148	50
Background	0.0395621	0.9428098	148	11400

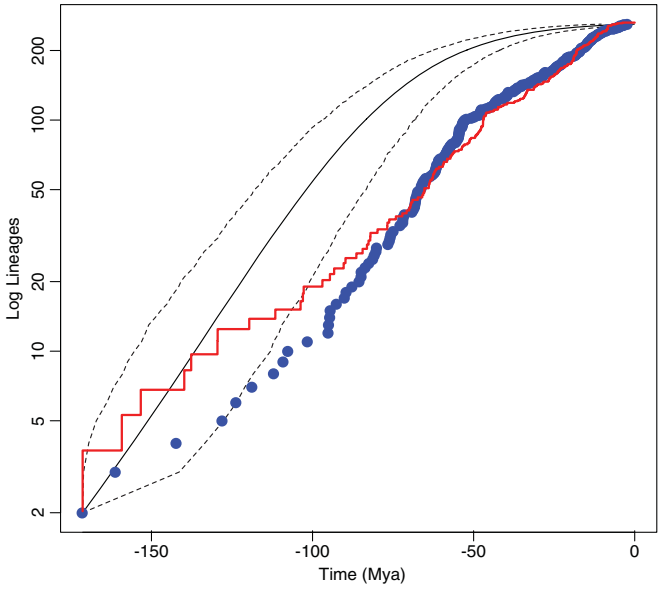

Figure 3. Log lineages-through-time plot of ant diversity based upon chronograms estimated with BEAST (see text for more detail). Blue dots indicate observed lineages-through-time for the chronogram included in Figure 2. Red line indicates lineages-through-time for divergence dating analysis with molecular data excluded. Black line and hatched black lines indicate mean and quantiles (0.025, 0.975) for simulated pure-birth trees, respectively. Time is presented in millions of years (Mya).

10 periods in which there was a significant change in the tempo, or rate, of diversification (Table 2; Fig. 2). In six of the cases we detected a significant rate increase with four cases of a significant rate decrease (Table 2). Results from our LTT analyses are shown in Figure 3. The LTT plot of our inferred tree (no duplicate species ML topology enforced and root node constrained to max 185 Mya) suggests that there was a shift in diversification in the modern ants shortly after 100 Mya and that our simulated PB trees do not fit our actual data very well. In addition, when we overlay our fossil only (molecular data excluded) BEAST analysis on our LLT plot, we see that although our fossil priors are influencing our inferred ages as demonstrated in the very similar ages for many of the major lineages (Table 1), our molecular data are still contributing informative data (Fig. 3).

BIOGEOGRAPHIC RANGE EVOLUTION

The results of our ancestral biogeographic range evolution analyses using ML in Lagrange (Ree et al. 2005; Ree and Smith 2008) were largely in congruence across the three analyses (unconstrained, dispersal only, dispersal and time) with higher variance for the number of possible inherited ranges for the more complex models. Across all analyses the inherited range splits for the major lineages presented in Figure 4 almost always shared the highest likelihood across all analyses. The most likely ancestral range inheritance scenarios were identical between the "unconstrained" and "dispersal only" analyses and only 20 of the 171 nodes

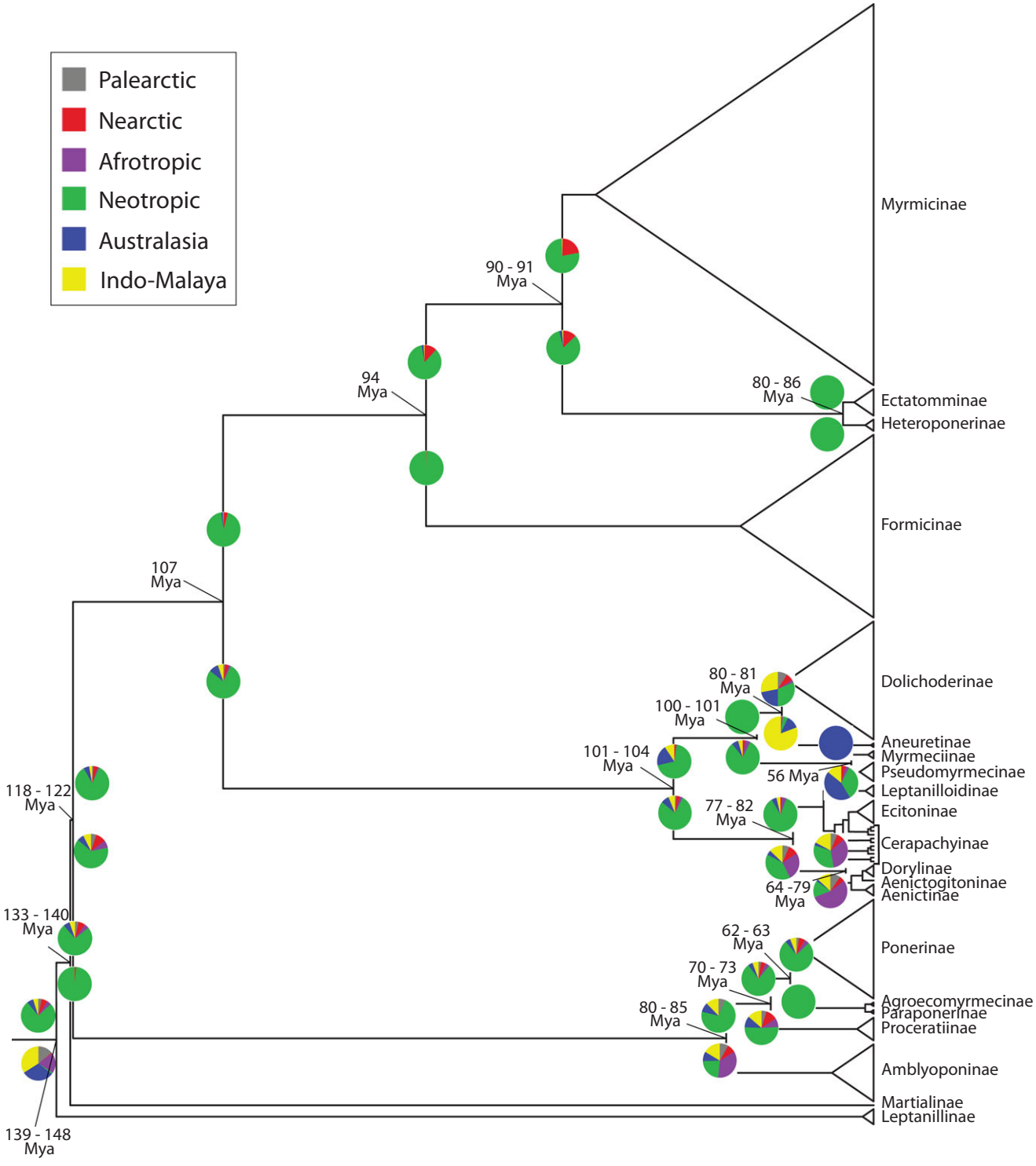

Figure 4. Biogeographic range evolution for the major ant lineages as inferred using a maximum likelihood framework (Lagrange) for the most complex model (dispersal and time). Subfamilies denoted on figure. Pie charts represent biogeographic ranges inherited by each descendant branch proportional to the marginal frequency an area was reconstructed. Only splits within two log-likelihood units of the maximum for each node are shown. Crown-group age estimates for inferred splits are shown with ages in millions of years ago (Mya). Colors refer to terrestrial ecozones as defined in the text: gray = Palearctic; red = Nearctic; purple = Afrotropic; green = Neotropic; blue = Australasia; yellow = Indo-Malaya.

differed in the most likely ancestral range across the more complex "dispersal and time" model, suggesting the reconstruction of these 20 nodes is ambiguous. Even across these 20 nodes the most likely areas from the first two analyses were always included in the inferred ancestral range in the "dispersal and time" model within two log-likelihood units of the maximum. The results of the "dispersal and time" analysis are presented visually using pie charts to represent biogeographic ranges inherited by each descendant branch proportional to the marginal frequency an area appears within two log-likelihood units of the maximum (Fig. 4).

Discussion

To fully understand the evolutionary history of life and the factors that may explain patterns of diversity, a well-resolved tree of life is paramount. With over 12,500 extant species we are far from having a completely resolved, species-level tree of life for the ants, although molecular phylogenetic analyses of the major ant lineages are providing a stable framework to understand the evolutionary relationships of the group (Brady et al. 2006; Crozier 2006; Moreau et al. 2006; Moreau 2009; this study). At the base of the ant tree of life, our analyses consistently recover the blind, subterranean Leptanillinae, and/or Martialinae as sister to all remaining ants (Figs. 1, S1) and hypothesis testing supports this relationship. This finding of the Leptanillinae and Martialinae as early lineages does not necessarily suggest that the earliest ant ancestors were hypogeic, below ground, cryptic forages with reduced-eyes as suggested by Brady et al. (2006), but more reasonable is that these lineages retreated underground after their evolutionary origin and changes to their morphology followed. Although many of the higher level relationships are now well resolved within the Formicidae, there still remains much work to do in resolving the generic and tribal-level boundaries across the family (Ward 2011) as the lack of monophyly for several diverse and commonly encountered genera attest including *Amblyopone*, *Aphaenogaster*, *Camponotus*, *Cerapachys*, *Messor*, *Monomorium*, *Pachycondyla*, *Pyramica*, *Strumigenys*, and *Tetraponera*.

Molecular clock analyses using 45 fossils as minimum age constraints recover an age of 139–158 Mya for the modern crown-group ants. Through our analyses using Bayesian molecular clock methods, which allow fossil constraints to have distributions as opposed to hard boundaries and do not require a fixed calibration point (BEAST; Drummond and Rambaut 2007), our data demonstrate that this method is less influenced by constraints such as a maximum age for the root node and are more stable across parameters and analyses than penalized likelihood. In addition, the Bayesian methods offer a promising opportunity to account for uncertainty in phylogenies by simultaneously estimating the topology and branch lengths (Drummond et al. 2006). Unlike penalized likelihood, which seems to rely heavily (at least in two previous ant studies; Brady et al. 2006; Moreau et al. 2006) on the fixed, maximum calibration point, we find that in implementing BEAST by enforcing or eliminating a maximum age on the root does not have a very large effect on the reconstructed age for the remaining lineages (Table 1). In addition, regardless of whether we constrained the topology or allowed BEAST to simultaneously estimate the topology, we arrive at very similar ages across the major lineages (Table 1). However, it appears that our fossil priors are still driving the posterior on divergence times for our data. Although of note, recent work by Heled and Drummond (2012) highlights potential problems with specifying prior calibration densities in the program BEAST. Although this issue can be addressed with smaller numbers of calibrations, implementing the method proposed by Heled and Drummond (2012) is not computationally possible (at this time) with the large number of nodes included here. Of additional note is that by comparing these results to previously published studies across lineages (Table 1), this variability in inferred ages between datasets and methods may suggest that using an age inferred in one divergence dating analysis as the foundation to calibrate another divergence dating analysis in groups without a fossil record may not be appropriate.

Modern ant diversity is highest in the world's angiosperm forests, in particular in the tropics (Wilson and Hölldobler 2005; Fisher 2010) and a latitudinal gradient in diversity has been observed in ants at both the generic (Dunn et al. 2010; McGlynn 2010; Guénard et al. 2012) and species (Kusnezov 1957; Jeanne 1979; Kaspari et al. 2004; Jenkins et al. 2011) taxonomic levels. Gymnosperm (conifer) dominated forests of both the Northern and Southern hemispheres are not without ants, but the complex forest litter and niches of modern angiosperm forests support far greater ant diversity. From the fossil record and current molecular clock analyses we know that angiosperm dominance occurred between the Upper Cretaceous and the Upper Eocene, 60–105 Mya (Crane et al. 1995; Schneider et al. 2004; Bell et al. 2005; Davis et al. 2005; Bell et al. 2010). This may provide an essential clue regarding the diversification of the ants: previous analyses estimate that much of the diversification of the major ant lineages occurred during the same time frame (Moreau et al. 2006; Moreau and Bell 2011; Table 1). Wilson and Hölldobler (2005) reached a similar conclusion based on the natural history of the modern ant fauna and knowledge of the fossil data. Many groups of insects (Farrell 1998; Wilf et al. 2000) and other animal and plant groups (Schneider et al. 2004; Roelants et al. 2007) experienced radiations corresponding to the expansion of the angiosperms, and ants appear to be among them (Moreau et al. 2006; Moreau and Bell 2011; this study). From these data and analyses, it also appears that many of the major ant lineages arose and diversified in this same time frame, although as we continue to collect more data, both in the amount of sequence data and the number of taxa included in these types of studies, as well as fossil information,

we hope that these estimates will converge. However, as we start to apply more and more complicated models of evolution to the estimation procedure, the more variance we may encounter in our estimates. This increase in variance, or certainty, in our estimates may make it difficult to reject certain hypothesis in favor of alternative ones.

Accounting for current species diversity within the ants using the MEDUSA method, which identifies multiple rate transitions, we observed that the majority of significant rate shifts within the ants occurred after Cretaceous/Tertiary (KT) boundary, throughout the Cenozoic (Fig. 2; Table 2). These diversification analyses identified 10 periods in which there was a significant change in the tempo, or rate, of diversification within the modern ants (Table 2; Fig. 2). In six of these cases we detected a significant rate increase with four cases of a significant rate decrease, although these do not correspond with where we see a shift in our LTT plot (Fig. 3), which does not account for current species diversity. Not surprisingly, in two cases in which a significant rate increase was detected these clades contain the two most species rich genera of ants (*Camponotus* and *Pheidole*). It is interesting that in two of the cases where a rate increase was detected these clades include ant genera that exhibit the "trap-jaw" syndrome, which may have allowed these groups to take advantage of novel prey (*Anochetus* + *Odontomachus* and *Pyramica* + *Strumigenys*). Also of note is that in two instances in which a significant rate decrease was detected these two clades represent three monotypic subfamilies. This initial diversification of the ants may have been followed by elevated extinction rates across the KT boundary, a hypothesis that has been suggested for other arthropod lineages (Wahlberg et al. 2009).

As the Neotropics are more plant species rich than the Paleotropics (Kier et al. 2005; Qian and Ricklefs 2008, and reference within) and previous work suggests that the ants diversified in response to the rise and dominance of the angiosperms (Moreau et al. 2006; Moreau and Bell 2011), this begs the question of whether the ants are tracking plant biogeography and diversification. Although the time frame in which many of these ant lineages are originating and diversifying does correspond with that of the origin of major angiosperm groups such as the rosids (Davis et al. 2005; Wang et al. 2009; Bell et al. 2010), the ages of the deeper nodes in the ant tree of life is somewhat problematic; their inferred ages are some 20–30 million years older than what some botanist believe to be the earliest tropical forests (see Davis et al. 2005). This would suggest that ants originated in some pretropical forest habitat and latter diversified and exploited the new niches that were made available once this tropical vegetation originated and expanded across the globe. Although tropical ant diversity (Fisher 2010) is correlated with the high angiosperm species richness of the tropics (Ricklefs and Renner 1994) and of the Neotropics in particular (Kier et al. 2005; Qian and Ricklefs 2008, and references within), we do not find any significant rate shifts within the ants that correspond to range shifts or to hypothesized movements of plant lineages into or out of the Neotropics. However, from our biogeographic ancestral range reconstruction, the Neotropics appears to have played an important role in the evolutionary history of the major ant lineages, as illustrated by the frequency and probability that this region was reconstructed across the ant tree of life (Fig. 4). Not surprisingly, the Neotropics are still home to a diverse assemblage of ant species with the Neotropics currently home to more genera (total number and endemic) and species than any other region (Guénard et al. 2010; Fisher 2010). These results suggest that the Neotropics have acted as a museum (as inferred in our biogeographic range reconstructions and high generic-level diversity), as well as a cradle for continued ant diversification (as suggested by the current high species richness of the region). This pattern of the Neotropics acting as both a museum and cradle of evolutionary novelty is not unique to the ants and has also been found in leaf beetles (McKenna and Farrell 2006) and birds (Diniz-Filho et al. 2007), and also more widely across the tropics in marine invertebrates (Jablonski 1993), Malpighiales plants (Davis et al. 2005), and passerine birds (Ricklefs 2006). Previous research suggests that the rise of the angiosperm dominated forests were an important factor in the diversification of the ants (Moreau et al. 2006; Moreau and Bell 2011) so it is not unexpected to find that ant evolution is tied to one of the tropical regions of the world. Our finding for ants of the Neotropics acting both as a museum, where ancient species persist along with ongoing diversification, and as a cradle, where species generation is high, when coupled with the fact that more endemic ant genera are found almost exclusively in tropical regions (Guénard et al. 2012) suggests that loss of species in these regions may disproportionately affect biodiversity of ants and many other species not just in the tropics, but also on a global scale.

Conclusions

This study provides to date the largest molecular phylogenetic analysis of ant relationships, large numbers of fossils and molecular data to arrive at an age for the evolution of modern crown-group ants, and the first attempt to infer the biogeographic history of this worldwide ecologically diverse group. Molecular data has provided a stable framework for the phylogenetic relationships of the major clades (Brady et al. 2006; Moreau et al. 2006; this study) with several surprising and unpredicted findings including the relationship of the subterranean leptanillines or martialines as sister to all extant ants. Coupling molecular phylogenetic data with the extensive fossil record of the ants suggest that the modern ants are 139–158 Mya. Our findings also suggest that Bayesian molecular clock methods such as BEAST (Drummond and Rambaut 2007), which allow fossil constraints to have distributions as opposed to hard boundaries and do not require a fixed calibration point,

may be less influenced by root node constraints and are more stable across parameters and analyses. However, using fossils as priors can have strong influence on the resulting posterior density for divergence times. Through ML biogeographic range reconstructions we find that the Neotropics were important in the early and continued evolutionary history of the ants, although shifts in the tempo of diversification within the ants do not correspond to historical biogeographic range shifts. This finding of a strong biogeographic history in the Neotropics coupled with current species richness of the region suggests that the Neotropics have acted as a museum and cradle for ant evolution.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Phylogeny of the ants estimated under Bayesian inference for the partitioned analysis with support values above 50% included.

Table S1. Minimum fossil calibrations used in this study.