Supporting Online Material for

Phylogeny of the Ants: Diversification in the Age of Angiosperms

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Supporting Online Material: Science manuscript, “Phylogeny of the Ants: Diversification in the Age of the Angiosperms” by Moreau et al.

**Taxon Sampling.**
The taxon sample represents all major lineages within Formicidae (Hymenoptera), including representatives of 19 of the 20 subfamilies. The analyses presented here include 149 specimens total. Included are 143 specimens representing 139 ant genera from 19 of the 20 currently recognized subfamilies within the Formicidae (Table S1). In addition, 6 Aculeata Hymenoptera outside of the Formicidae were included as outgroups (Vespidae, Bradynobanidae, Pompilidae, Mutillidae, Sphecidae, and Apidae) (Appendix S1). Sequence for *Apis mellifera* was obtained from GenBank. All sequences have been deposited in GenBank (Accession Numbers DQ352864 – DQ353694) (Appendix S2); collection information for each specimen is available from the first author.

**Table S1.** Number of genera represented in this study; extant; percent genera represented in this study.

<table>
<thead>
<tr>
<th>Clade</th>
<th>No. Genera in this Study</th>
<th>No. Extant Genera</th>
<th>Percent present in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incertae Sedis</td>
<td>0</td>
<td>3</td>
<td>0%</td>
</tr>
<tr>
<td>Myrmicinae</td>
<td>52</td>
<td>139</td>
<td>37%</td>
</tr>
<tr>
<td>Formicinae</td>
<td>25</td>
<td>49</td>
<td>51%</td>
</tr>
<tr>
<td>Ectatomminae</td>
<td>4</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Heteroponerinae</td>
<td>1</td>
<td>3</td>
<td>33%</td>
</tr>
<tr>
<td>Dolichoderinae</td>
<td>16</td>
<td>23</td>
<td>70%</td>
</tr>
<tr>
<td>Aneuretinae</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Pseudomyrmecinae</td>
<td>2</td>
<td>3</td>
<td>67%</td>
</tr>
<tr>
<td>Myrmeciniinae</td>
<td>1</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>Aenictinae</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Dorylinae</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Ectoninae</td>
<td>4</td>
<td>5</td>
<td>80%</td>
</tr>
<tr>
<td>Cerapachyinae</td>
<td>3</td>
<td>6</td>
<td>50%</td>
</tr>
<tr>
<td>Leptanilloidinae</td>
<td>1</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>Ponerinae</td>
<td>12</td>
<td>26</td>
<td>46%</td>
</tr>
<tr>
<td>Agroecomyrmecinae</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Paraponerinae</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Amblyoponinae</td>
<td>8</td>
<td>10</td>
<td>80%</td>
</tr>
<tr>
<td>Proceratiinae</td>
<td>3</td>
<td>3</td>
<td>100%</td>
</tr>
<tr>
<td>Leptanillinae</td>
<td>2</td>
<td>5</td>
<td>40%</td>
</tr>
<tr>
<td>Formicoid Clade</td>
<td>112</td>
<td>239</td>
<td>47%</td>
</tr>
<tr>
<td>Poneroid Clade</td>
<td>25</td>
<td>41</td>
<td>61%</td>
</tr>
<tr>
<td>Leptanilloid Clade</td>
<td>2</td>
<td>5</td>
<td>40%</td>
</tr>
<tr>
<td>Formicidae</td>
<td>139</td>
<td>288</td>
<td>48%</td>
</tr>
</tbody>
</table>

**DNA isolation.**
Field collections were made in 95% EtOH and kept at -20°C in the laboratory until the time of DNA extraction. Total genomic DNA was isolated by grinding either an entire ant specimen or part of a specimen in lysis buffer with a Teflon grinding implement, followed by purification
using the DNeasy™ Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer’s protocols.

**PCR Amplification.**
For each specimen, five nuclear genes and one mitochondrial gene were amplified via PCR when possible (S1, S2) using published and/or optimized primers (Table S2). Double-stranded DNA was amplified in 25 µL volume reactions: 16.15 µL ultra pure (HPLC quality) water, 2.5 µL 10X buffer, 1.5µL 25mM MgCl₂, 0.25 µL 100mM dNTP, 1.2 µL of each primer (10mM), 1 µL DMSO, and 0.2 µL Taq DNA Polymerase (Qiagen Inc., Valencia, CA). All reactions were initially denatured at 94°C for two minutes in a MJ Dyad Thermal Cycler (MJ Research, Waltham, MA), then subjected to 35 cycles of 60s at 94°C denaturation, 60s at 45°C - 56°C (annealing temperature depended on gene amplified) for annealing, and 2 min at 72°C extension.

**Table S2.** Primer sequences for amplification and sequencing of genes used in this study.

<table>
<thead>
<tr>
<th>Primer Location</th>
<th>Primer Name</th>
<th>Primer Utility</th>
<th>Sequence</th>
<th>Primer Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA cox1</td>
<td>LCO1490</td>
<td>Amplification/Sequencing</td>
<td>5'-GGTCAACAATACTATAAGATATTGG-3'</td>
<td>S3.</td>
</tr>
<tr>
<td></td>
<td>HCO2198</td>
<td>Sequencing</td>
<td>5'-TAAAACCTAGGGTGACCAAAATCA-3'</td>
<td>S3.</td>
</tr>
<tr>
<td></td>
<td>Jerry</td>
<td>Sequencing</td>
<td>5'-CAAATTATTGATTTTGG-3'</td>
<td>S4.</td>
</tr>
<tr>
<td></td>
<td>Ben</td>
<td>Amplification/Sequencing</td>
<td>5'-GCTACTACATATAATGTATCTAG-3'</td>
<td>S5.</td>
</tr>
<tr>
<td>nRNA 18S</td>
<td>rc18A</td>
<td>Amplification/Sequencing</td>
<td>5'-TGTTGATCTCCAGTAG-3'</td>
<td>S6.</td>
</tr>
<tr>
<td></td>
<td>18N'</td>
<td>Amplification/Sequencing</td>
<td>5'-CCCCTGAATTTAAGCAT-3'</td>
<td>S7.</td>
</tr>
<tr>
<td>nRNA 28S</td>
<td>Mo6</td>
<td>Amplification/Sequencing</td>
<td>5'-CCCCTGAATTTAAGCAT-3'</td>
<td>S7.</td>
</tr>
<tr>
<td></td>
<td>D2B</td>
<td>Sequecing</td>
<td>5'-GTCCGGTGTGCTGAGAGTGC-3'</td>
<td>S8.</td>
</tr>
<tr>
<td></td>
<td>D2B-r</td>
<td>Sequencing</td>
<td>5'-GCACCTCAAGAAACCGCAGC-3'</td>
<td>S8.</td>
</tr>
<tr>
<td></td>
<td>D3A</td>
<td>Sequencing</td>
<td>5'-GACCGGTCTTTGAACACAGA-3'</td>
<td>S8.</td>
</tr>
<tr>
<td></td>
<td>D3A-r</td>
<td>Sequencing</td>
<td>5'-TCGGTGTTCAAGACCAGTGC-3'</td>
<td>S8.</td>
</tr>
<tr>
<td>nDNA Wingless</td>
<td>Wg578F</td>
<td>Amplification/Sequencing</td>
<td>5'-TGCAACGTAARACTYTCTGAGGATGCG-3'</td>
<td>S9.</td>
</tr>
<tr>
<td>(Wg)</td>
<td>Wg1032R</td>
<td>Amplification/Sequencing</td>
<td>5'-ACYTCGACAGCAGCAGGAA-3'</td>
<td>S10.</td>
</tr>
<tr>
<td>nDNA Abdominal-A</td>
<td>ant-M</td>
<td>Amplification/Sequencing</td>
<td>5'-CGGACCACGCGATAGTAGCAGAATTTG-3'</td>
<td>S11.</td>
</tr>
<tr>
<td>(abd-A)</td>
<td>ant-J</td>
<td>Amplification/Sequencing</td>
<td>5'-GATGTTCAAGAGCTATGCAAGGAGT-3'</td>
<td>S11.</td>
</tr>
<tr>
<td>nDNA LW Rhodopsin</td>
<td>LR143F</td>
<td>Amplification/Sequencing</td>
<td>5'-GACAATGKCCACCRGARATGCT-3'</td>
<td>S9.</td>
</tr>
<tr>
<td>(LR)</td>
<td>LR639ER</td>
<td>Amplification/Sequencing</td>
<td>5'-YTTACCGRTCCATCCRAACA-3'</td>
<td>S9.</td>
</tr>
</tbody>
</table>

**Sequencing.**
All sequencing was done using dye terminator cycle sequencing following the protocol specified by the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Revision B, August 1995, Perkin-Elmer, Norwalk, CT). Primers used for amplification served as sequencing primers. Additional internal primers were designed for sequencing purposes (Table S2) to provide overlapping sequence coverage for the entire region for selected genes. All samples
were sequenced in both directions. Cycle sequencing reactions were performed in 12 µL reactions: 1.5 µL ABI Prism® BigDye™ v3.1 (Applied Biosystems Inc., Foster City, CA), 1.0 µL 5x buffer (buffer: 400 mM Tris at pH 9.0 and 10mM MgCl₂), and 0.33 µL each (10 µM) primer. The remainder of the mixture was composed of ultra pure water and template to give 50-90 ng of template DNA in each reaction. Cycle sequence reaction parameters contained an initial denature step of 94°C for 2 min, followed by 25 cycles of 10s at 94°C denaturation, 5s at annealing (temperature varied for gene regions) and 4 min at 60°C (MJ Dyad Thermal Cycler, MJ Research, Waltham, MA).

Sequence Alignment.
After sequences were collected, they were analyzed and initially aligned using the computer programs Sequencing Analysis 3.7 (ABI Prism™ 2001) and Sequencher 4.2 (GeneCodes 2003), respectively. Conserved regions were identified and aligned, and gaps assigned to minimize changes using ClustalX 1.9a169 (S12). For the four protein-encoding genes, the inferred amino acid sequences were used which allowed for comparatively uncomplicated alignment. To insure the most appropriate alignment of the ribosomal genes, the aligned data set was finally viewed and further manually aligned using MacClade 4.06 (S13), with highly ambiguously aligned regions removed. All data sets analyzed for this study are available from TreeBASE (S14) or by request from the first author.

Preliminary sequence analysis.
Base composition bias was calculated (S15) for the entire fragment. A value of zero indicates no bias and a value of one indicates complete bias. An extreme overabundance of one nucleotide state can increase the tendency for those sites to become saturated (S15). In addition, a strongly skewed mutation bias can violate the assumption in maximum parsimony analysis that there is an equal probability of change at all sites (S16). The heterogeneity chi square test in PAUP* 4.0b10 (S17) was used to test for bias among taxa.

Phylogenetic analysis.
To infer relationships among the ants, several phylogenetic analyses were performed using PAUP* 4.03b10 (S17), PhyML v2.4.4 (S18) and MrBayes v3.1.1 (S19). A variety of model based methods, in addition to maximum parsimony, were employed to infer phylogenetic relationships. Parsimony has been shown to be inconsistent under certain situations (S20), particularly when dealing with certain types of molecular sequence data (S21-S23), so maximum likelihood approaches were also used. To insure testing of the monophyly of the Formicidae, we only constrained *Apis* as the outgroup.

**Maximum parsimony** - parsimony searches were performed using the random stepwise addition option of the heuristic search for 300 replicates with tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, and equal weighting of all characters. Several maximum parsimony analyses were conducted: one including all sites in the alignment (the “complete” data set), the “all genes” data set with 47 taxa removed that were missing one or more gene in the matrix, and finally a search for each of the six individual genes. 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 (S24,
were executed by using the closest stepwise addition of the heuristic search for 300 replicates.

**Maximum likelihood** - In order to evaluate the fit of the data, a maximum likelihood analysis was conducted using again the complete data set, as well as the “all genes” data set, and a search for each of the six individual genes with both PAUP*4.03b10 (S17) and PhyML v2.4 (S18). Maximum likelihood methods were employed to infer both tree topologies and branch lengths for six regions. For each data set, a series of likelihood ratio tests (LTRs) was performed to select a model for maximum likelihood searches using PORN* (S26). In PAUP*, parameters for the searches were estimated on a tree topology obtained in a maximum likelihood search using a Jukes-Cantor (S27) (JC) model of sequence evolution. Parameters for each model were then fixed, and heuristic searches were run using 100 random taxon additions and tree-bisection-reconnection (TBR) branch swapping. A final round of searching entailed estimating all model parameters simultaneously using the tree obtained in the previous step as the starting tree and nearest-neighbor interchange (NNI) branch swapping. Following each search, a LRT was used to test for departure from clock-like evolution in the molecular sequence data (S28). Bootstrap tests were performed using PhyML for 300 replicates.

Competing tree topologies based on previous taxonomic definitions of groups was compared using the Shimodaira-Hasegawa test to test for significant difference in tree lengths. This test was performed using RELL with 1,000 bootstrap replicates, and the results were evaluated as a one-tailed test.

**Bayesian inference** - Likelihood analyses were also performed in a Bayesian framework using the program MrBayes v3.1.1 (S19), with model parameters being estimated during the run, and using the default value of four Markov chains. Multiple chains can assist in more easily traversing tree-space and help avoid entrapment in local topological optima. The “temperature” parameter was set to 0.2, resulting in incremental heating of each chain. Higher temperature values result in greater differences in heating between chains, and hotter chains are less constrained by likelihood scores in moving through tree-space (S29). The Monte Carlo Markov chain (MCMC) length was 10,000,000 generations, and we sampled the chain every 1000 generations with a burn-in of 10,000 cycles. Bayesian posterior probabilities (bpp) were estimated as the proportion of trees sampled after burn-in that contained each of the observed bipartitions (S30). A single GTR+Γ model of sequence evolution was assumed to underlie all gene regions.

In addition, an analysis was conducted using “mixed models” of molecular sequence evolution. In these analyses, each gene region was assigned its own GTR+Γ model. This approach was only done on the “complete” data set.

**Fossil constraints.**
The use of fossils in concert with molecular data can take two forms: (1) fossils can serve as fixed “calibration” points used to calculate absolute branching times, or (2) they can serve as maximum or minimum age “constraints” (S31). For this study, we selected 43 fossils that we believe can be confidently placed in the ant tree to use as separate minimum age constraints (Table S3). We used only the oldest confident member of extant genera or subfamilies to calibrate divergence times of modern crown-group ants, excluding stem-group ants such as the Sphecomyrminae. Engel and Grimaldi (S32) recently provided an updated list of Cretaceous ant
genera, including some that they found to be of uncertain subfamily placement, which we also excluded; we further excluded those that have been subsequently found to be of ambiguous position (S33).

**Table S3.** Fossil calibration points used in this study as minimum age constraints. Fossil dates with asterisk indicate that molecular clock analyses were done with both lower and upper dates to insure confidence in dating of fossils.

<table>
<thead>
<tr>
<th>Node / Taxon</th>
<th>Oldest Fossil (Ma)</th>
<th>Fossil Locality</th>
<th>Fossil Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptogenys</td>
<td>15.5</td>
<td>Shanwang Formation, China</td>
<td>S34.</td>
</tr>
<tr>
<td>Myopopone</td>
<td>15.5</td>
<td>Shanwang Formation, China</td>
<td>S34.</td>
</tr>
<tr>
<td>Acropyga</td>
<td>15.0 - 20.0 *</td>
<td>Dominican Amber, Dominican Republic</td>
<td>S35.</td>
</tr>
<tr>
<td>Azteca</td>
<td>15.0 - 20.0 *</td>
<td>Dominican Amber, Dominican Republic</td>
<td>S36.</td>
</tr>
<tr>
<td>Cephalotes</td>
<td>15.0 - 20.0 *</td>
<td>Mexican Amber, Mexico</td>
<td>S37.</td>
</tr>
<tr>
<td>Discophytrea</td>
<td>15.0 - 20.0 *</td>
<td>Mexican Amber, Mexico</td>
<td>S38.</td>
</tr>
<tr>
<td>Neivamyrnex</td>
<td>15.0 - 20.0 *</td>
<td>Dominican Amber, Dominican Republic</td>
<td>S36.</td>
</tr>
<tr>
<td>Odontomachus</td>
<td>15.0 - 20.0 *</td>
<td>Dominican Amber, Dominican Republic</td>
<td>S39.</td>
</tr>
<tr>
<td>Pyramica</td>
<td>15.0 - 20.0 *</td>
<td>Dominican Amber, Dominican Republic</td>
<td>S39.</td>
</tr>
<tr>
<td>Trachymyrnex</td>
<td>15.0 - 20.0 *</td>
<td>Dominican Amber, Dominican Republic</td>
<td>S40.</td>
</tr>
<tr>
<td>Crematogaster</td>
<td>28.4 - 33.9 *</td>
<td>Sicilian Amber, Italy</td>
<td>S41.</td>
</tr>
<tr>
<td>Podomyrma</td>
<td>28.4 - 33.9 *</td>
<td>Sicilian Amber, Italy</td>
<td>S41. &amp; S42.</td>
</tr>
<tr>
<td>Pheidole</td>
<td>34.0</td>
<td>Florissant Formation, USA</td>
<td>S43.</td>
</tr>
<tr>
<td>Paggonomyrnx</td>
<td>34.0</td>
<td>Florissant Formation, USA</td>
<td>S43.</td>
</tr>
<tr>
<td>Agroecomyrmecinae</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S44.</td>
</tr>
<tr>
<td>Anonychomyrma</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Aphaenogaster</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Camponotus</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Cerapachys</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Formica</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Iridomyrnx</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Lasius</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Monomorium</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Myrmica</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Oligomyrnx</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Plagiolepis</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Proceratiniae</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S46.</td>
</tr>
<tr>
<td>Rhytidoponera</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Solenopsis</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Stenamma</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Tetramorium</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Tetraponera</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Vollenhovia</td>
<td>44.1</td>
<td>Baltic Amber</td>
<td>S45.</td>
</tr>
<tr>
<td>Myrmicinae</td>
<td>52.0</td>
<td>Hat Creek Amber, Canada</td>
<td>S47.</td>
</tr>
<tr>
<td>Tapinoma</td>
<td>52.0</td>
<td>Hat Creek Amber, Canada</td>
<td>S48.</td>
</tr>
<tr>
<td>Dolichoderus</td>
<td>48.5 - 53.5 *</td>
<td>Green River Formation, USA</td>
<td>S49.</td>
</tr>
<tr>
<td>Pachycondyla</td>
<td>48.5 - 53.5 *</td>
<td>Green River Formation, USA</td>
<td>S49.</td>
</tr>
<tr>
<td>Myrmecinae</td>
<td>54.5</td>
<td>Ølst Formation, Denmark</td>
<td>S33.</td>
</tr>
<tr>
<td>Ponerinae</td>
<td>60.0</td>
<td>Sakhalin Amber, Russia</td>
<td>S50.</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Age (Ma)</td>
<td>Location</td>
<td>Notes</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------</td>
<td>---------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Dolichoderinae</td>
<td>79.0</td>
<td>Canadian Amber, Canada</td>
<td>S32. &amp; S51.</td>
</tr>
<tr>
<td>Ectatomminae</td>
<td>79.0</td>
<td>Canadian Amber, Canada</td>
<td>S32. &amp; S51.</td>
</tr>
<tr>
<td>Formicinae</td>
<td>92.0</td>
<td>New Jersey Amber, USA</td>
<td>S52.</td>
</tr>
<tr>
<td>Aneuretinae</td>
<td>100.0</td>
<td>Burmese Amber, Myanmar</td>
<td>S53. &amp; S54.</td>
</tr>
</tbody>
</table>

We follow the age boundaries and terminology as currently set by the International Commission on Stratigraphy (2004) (S55). Where the stratigraphic position of a fossil occurrence is not well resolved within a formation, we have used both the minimum and maximum ages of that formation in our analyses. Exceptions are as noted below (Sakhalin amber, Baltic amber).

**Ages of localities used in analyses or mentioned in the text.**

**Khentiana River.** (Emanra Fm, Russia). The oldest known armanines are from the middle Albian Khentiana River locality, Emanra Formation, Russia (S57). Grimaldi and Engel (S56) cited this locality as ~125 Ma.

**French amber.** (Charente-Martime, France). Considered late Albian, ~100 Ma, and close in age to Burmese amber (S32, S57).

**Burmese amber.** (Myanmar). Late Albian, but possibly Cenomanian (S32, S56, S58).

**New Jersey amber.** USA. Late Cretaceous, Turonian, ~92 Ma (S52).

**Canadian amber.** (Cedar Lake, Manitoba; Medicine Hat, Alberta). The Alberta occurrence is from the upper part of the Judith River Group of the Foremost Formation, about 78-79 Ma; the Cedar Lake, Manitoba locality is considered secondary deposition of the same material (S59).

**Sakhalin amber.** (Russia). Collected on the shore of Sakhalin Island; recently found in situ in the Paleocene Lower Due Formation (S60). As neither a date for the amber within the Paleocene (55.8 ± 0.2 to 65.5 ± 0.3 Ma), nor upper and lower ages of the Due Fm are provided, we employ a middle Paleocene value of 60 Ma.

**Ølst Formation.** (Northern Denmark). “Mo-clay” in part. The myrmeciine ants are from the Stollenklint Clay member, above the Paleocene/Eocene boundary $\delta^{13}$C excursion (S33). An Ypresian age of 54.52 ± 0.05 Ma has been determined for volcanic ash layer –17 by the Ar$^{40}$-Ar$^{39}$ method (S61), above the ant-bearing beds.

**Hat Creek amber.** (Okanagan Highlands; British Columbia, Canada). The Hat Creek locality is no older than Eocene by palynology, and a minimum K-Ar age of 52.2 ± 1.4 Ma has been provided for rhyolite above the amber-bearing coal (S62). Reed (S63) suggests, however, that the rhyolite may have been secondarily superimposed. Investigation is ongoing (S64).

**Green River Formation.** Colorado, USA. Lacustrine shales deposited over ~5 Ma, from ~53.5 to 48.5 Ma, or mid-Ypresian (Early Eocene) into (barely) the earliest Lutetian (early Middle Eocene) (S65-S66).
**Baltic amber.** The precise age of Baltic amber within the Eocene remains controversial. The majority of the amber is found in the “Blue Earth”, dated 44.1 ±1.1 Ma (middle Lutetian) by K-Ar decay (S67). Some, but not as much amber comes from the "Wilde Earth", a member below the Blue Earth. A sample from the upper Wilde Earth was dated at 47.0 ±1.5 Ma (earliest Lutetian) (S67). These ages were determined from glauconites, at times problematic (S68-S69). Re-deposition adds further uncertainty (S70). The amber-producing forest may have persisted for an extended period, perhaps as long as ten Ma (S70, S71). A Priabonian determination of the Blue and Wilde Earths is supported by marine microfossil biostratigraphy (S72, S73). Kaplan et al. (S74) find of the glauconite age of 37.7 ±3 Ma (around the Bartonian/Priabonian boundary, or earliest Priabonian). We use the 44.1 ±1.1 Ma age here, as do various recent authors (S56), bearing in mind that this further represents a somewhat middle estimate of the range discussed above, and that no useful upper and lower bounds are determined, as for, e.g., the Green River Fm.

**Florissant.** Colorado, USA; Late Eocene (Priabonian): 34.07 ± 0.10 Ma by Ar$^{40}$-Ar$^{39}$ dating (S75).

**Sicilian amber.** Oligocene, perhaps early Oligocene (S42). We employ the age range of the first half of the Oligocene, the Rupelian age (33.9 ±0.01 Ma to 28.4 ±0.01 Ma).

**Dominican amber.** Early Miocene, 15-20 Ma (S76).

**Mexican amber** (Chiapas amber). Likely equivalent to Dominican amber (S77).

**Shanwang Formation, China.** Mid-Miocene, 15.5 Ma (S34) (J. Zhang, personal communication).

**Molecular clock analysis: Penalized likelihood (PL).** Penalized likelihood (S78) (PL) is a semi-parametric smoothing method. Penalized likelihood assumes that there is an autocorrelation of substitution rates and attempts to minimize rate changes between ancestral/descendant branches on a tree (i.e., at the nodes). PL attempts to combine the statistical power of parametric methods (models of molecular evolution) with the robustness of nonparametric methods. A smoothing parameter can vary from very small, in which case each branch of the phylogeny has a different substitution rate (saturated model), to very large, in which parameters are essentially clock-like. The crux of the penalized likelihood method is determining the optimal smoothing level. The program r8s v1.7 (S79) implements a data-driven cross-validation procedure that systematically prunes terminals from the tree, then estimates parameters from the submatrix and a given smoothing value. It then tries to predict the data for pruned taxa using the estimated parameters. Finally, it calculates a chi-squared error associated with the difference between the predicted and observed data. The optimal smoothing level is chosen as the one that minimizes the chi-squared error (S76). Standard deviations on age estimates where calculated via non-parametric bootstrapping.

**Molecular clock analysis: A heuristic rate smoothing (AHRS).**
A heuristic rate smoothing (AHRS) method, proposed by Yang (S80), involves a three-step process to estimate divergence times. First, branch lengths are estimated on each gene tree without the clock. Next, heuristic rate smoothing is used to estimate one set of divergence times for the nodes in the species tree and as many sets of branch rates as the number of loci. Each branch in the gene tree is assigned an estimated rate. A smoothing parameter called \( \nu \) is also estimated for each locus. The rate estimation is achieved by minimizing the discrepancy between the predicted branch lengths and the estimates obtained in the first step, and by minimizing rate changes over time. The estimated rates for branches in the gene trees are then collapsed into a few branch rate categories. Finally, divergence times are estimated simultaneously with the rates for branch rate groups. All AHRS analyses were performed with the computer software PAML v3.15 (S81). In these analyses, fossils were used as fixed calibration points, as opposed to minimum age constraints. Standard deviations were calculated with PAML.

**Molecular clock analysis: Bayesian relaxed clock (BRC) using Markov chain Monte Carlo.**
Bayesian methods (S82-S84) that relax a strict molecular clock were also used to estimate divergence times using MULTIDIVTIME (available from J. Thorne, North Carolina State University). This parametric approach relaxes the assumption of a strict molecular clock with a continuous autocorrelation of substitution rates across the phylogeny, and allows the use of several calibrations/time constraints.

Divergence date estimation with MULTIDIVTIME involved two steps. First, ESTBRANCHES was run to estimates branch lengths from the data and a fixed tree topology using the F84 (S85, S86) model of sequence evolution. This is currently the most parameter rich model the program allows. This model does allow rates to vary among sites following a discrete gamma distribution with four rate categories (S87) along with their variance-covariance matrix. Parameters for the F84 + \( \Gamma \) model were estimated using the BASEML program in PAML (S80).

Next, the root (in our case, *Apis mellifera*) is pruned from the tree and MULTIDIVTIME is used to estimate the prior and posterior ages of branching events, their standard deviations, and the 95% credibility intervals via Markov chain Monte Carlo. The Markov chain was run for 1,000,000 generations and sampled every 100 generations after an initial burn-in period of 10,000 cycles. To check for convergence of the MCMC, analyses were run from at least two different starting points.

The following prior distributions were used in these analyses: 225 Ma (SD = 25 Ma) for the expected time between tip and root if there were no constraints; 0.0026 (SD = 0.0026) substitutions per site per million year for the rate of the root node; 0.004 (SD = 0.004) for the parameter that determines the magnitude of autocorrelation per million years; and 300 Ma for the largest value of the time unit between the root and the tips.

**Estimating Rates of Diversification.**

Diversification rates (speciation minus extinction) were investigated using lineage-through-time (LTT) plots and alternative models of diversification were tested using the statistical approach of Paradis (S88-S90). The ultra-metric tree obtained from the penalized likelihood analyses was used to calculate proportional lineages-through-time (LTT) plots for ants. Numbers of lineages were tallied at sequential time points (10Myr intervals) and are presented as proportions (%) of the number of ants.

Given a birth-death model of diversification, if rates of speciation are uniform throughout
the history of the clade, the lineages-through-time plot is expected to be linear. An increase in the rate of speciation or a constant rate of background extinction will cause an upturn in the plot, giving a steeper slope. Alternatively, a decrease in the rate of speciation or an increase in the rate of extinction will cause a leveling of the slope (S91). Distinguishing between speciation and extinction using lineages-through-times plots is often difficult, if not impossible (S92). However, we used the pattern of the lineages-through-time plot to graphically assess whether there is the appearance of an increased rate of diversification during the Cretaceous.

To statistically test for an increased rate of diversification in crown group ants during the Cretaceous, we used methods proposed by Paradis (S88-S90). These methods use survival models to examine divergence times within a clade of interest. Using estimates of divergence times, a rate of diversification (\(\delta\)) is estimated using a maximum-likelihood approach (S88). For each node in the phylogeny, an instantaneous diversification rate \(\delta(t)\), with components of instantaneous speciation \(\sigma(t)\) and instantaneous extinction \(\varepsilon(t)\) is assumed. Using estimated divergence times from penalized likelihood estimations, the presence of variation in the rate of diversification within crown group ants was tested.

The computer program APE v1.4 (S93) allows the fitting of three separate models using maximum likelihood. Model A assumes a constant rate of diversification (\(\delta\)) throughout time, model B allows \(\delta\) to vary through time, and model C assumes two different rates of diversification before and after a specified breakpoint in time (S88). Model B assumes a gradual change in \(\delta\) through time, and the critical parameter is \(\beta\). If \(\beta < 1\), then \(\delta\) is decreasing; if \(\beta > 1\), \(\delta\) is increasing through time in the clade (S88). APE calculates likelihoods and AIC (Akaike information criterion) values for the fit of the divergence times to each of the three competing models. Because model A is nested in models B and C, they can be compared using a likelihood ration test (LRT). Models B and C are not nested, therefore AIC can be used to reject one model over the other. The model with the lowest AIC is the model selected as the one best describing the temporal variation of divergence times. Based on the expectation of the Cretaceous diversification model, we set the breakpoint for model C to 100 ma. If an increase in the rate of diversification during the Cretaceous is projected, rejection of model A in favor of model B or model C is expected. If any increase in the rate of diversification occurred after 100 mya, AIC should favor model C over model B.

RESULTS & DISCUSSION

Simple Sequence Statistics.
This study produced a final aligned 4572 base pair (bp) fragment with most taxa represented for a the following six gene regions: rDNA 18S (772 bp), rDNA 28S (1455 bp), nDNA Abdominal-A (abd-A) (672 bp), nDNA Wingless (Wg) (424 bp), nDNA Long-Wavelength Rhodopsin (LR) (598 bp), and mtDNA Cytochrome Oxidase I (cox1) (1044 bp). The aligned fragment contained 452 sites that were variable (9.8%) and 1744 sites that were phylogenetically informative (38.1%). Examinations of base composition in the data sets resulted in the following for the entire data set: A: 0.23732; C: 0.26721; G: 0.24879; T: 0.24688. A Chi-square test for homogeneity of base frequency among taxa was non-significant when all characters were included resulting in a value of 2029.463939 with 444 degrees of freedom (P < 0.001). When uninformative sites were eliminated, the Chi-square value was 3303.15169 with 444 degrees of freedom, and once again the test for homogeneity of base frequency among taxa remained non-significant (P < 0.001). This data set exhibited low base composition bias for the entire
alignment, as well as, for each individual gene. The homogeneity test suggests that none of the sequence, either as an entire alignment or as individual partitions, was heterogeneous.

To insure ambiguously aligned regions where not contributing misleading information to our dataset, we excluded 261 bp of sequence data from the rDNA 28S gene and 132 bp of sequence data from the intron of nDNA LR. This resulted in the final aligned 4572 base pair fragment, which was used in all analyses.

**Parsimony phylogenetic analyses.**
The maximum parsimony analysis of all characters resulted in 13 equally parsimonious trees (Length = 27,706). Bootstrap values are presented on the maximum likelihood topology Fig. S1. To examine the affect of missing data for taxa on the analysis, 47 taxa were removed, which created the “all genes” data set of 102 taxa. The parsimony analysis with missing data taxa removed resulted in 5 equally parsimonious trees (L = 20,858). The consensus of this tree is virtually the same as for the complete data set. The “all genes” data set consensus still maintains the formicoid clade. The key differences present in the “all genes” data set consensus tree relative to the complete data set tree consists of a lack of resolution for certain taxa, but not an entirely different tree, suggesting that the missing data for taxa are not contributing a disproportionate effect on the topology. The major effect produced by removing the missing data for taxa was to lower the bootstrap support slightly at some of the nodes, which is expected since fewer characters are present in the “all genes” data set. Although the inclusion of all the taxa does not appear to have any adverse effects on recovering the phylogeny, all subsequent analyses were conducted using both all the complete data set and the “all genes” data set.

**Maximum likelihood phylogenetic analyses.**
The maximum likelihood model determined using the LRT on the complete dataset, the “all genes” dataset, as well as, for each individual gene partition suggested that the best model for these data was the GTR +Γ. The maximum likelihood search in PAUP* and PhyML using this model resulted in one maximum likelihood tree for the complete dataset with a –ln L = 121467.96248 (Fig.S1) when all characters were included. The parameter values as estimated from this tree were: A⇔C: 2.16670, A⇔G: 6.61745, A⇔T: 5.60729, C⇔G: 3.13578, C⇔T: 17.08553, G⇔T: 1.0 for the GTR model, estimated base composition was A = 0.288251, C = 0.222196, G = 0.180652, T = 0.308901, α = 0.328730 for the Γ distribution. Bootstrap values are presented on the maximum likelihood topology (Fig. S1).

Maximum likelihood was also used to test for a clocklike evolution (i.e., rate constancy among lineages). The molecular clock tree produced with the same parameter estimates above gave a likelihood score of –ln L = 122,178.92442, which indicates that the molecular clock should be rejected ($\chi^2 = 1,421.92$, df = 147, P < 0.0001). The maximum likelihood search on the “all genes” data set in PAUP* and PhyML, also resulted in one maximum likelihood tree with a likelihood score of –ln L = 93,110.80514. The parameter values as estimated from this tree were: A⇔C: 2.27319, A⇔G: 7.56729, A⇔T: 10.21440, C⇔G: 2.09047, C⇔T: 17.50772, G⇔T: 1.0 for the GTR model, estimated base composition was A = 0.23935, C = 0.26370, G = 0.24482, T = 0.25213, α = 0.28629 for the Γ distribution.

After constraining the Cerapachyinae as a monophyletic lineage, we used the Shimodaira-Hasegawa test with 1,000 RELL bootstraps (-lnL 119720.61675 present study; 119813.25331 Cerapachyinae constrained as monophyletic lineage; difference in –lnL 92.63656) and found the constraint to be significantly different (P = 0.047).
Bayesian Inference.
The maximum likelihood analysis of the complete dataset in MrBayes v3.1.1 using the GTR +$\Gamma$ model of sequence evolution resulted in a sample of trees with a mean likelihood score of $-\ln L = 121,320.867$. The parameter mean values as estimated from this sample were: $A\leftrightarrow C$: 0.06005, $A\leftrightarrow G$: 0.202, $A\leftrightarrow T$: 0.155, $C\leftrightarrow G$: 0.09277, $C\leftrightarrow T$: 0.459, $G\leftrightarrow T$: 0.03084 for the GTR model, estimated base composition was $A = 0.287, C = 0.228, G = 0.168, T = 0.317$, $\alpha = 0.325$ for the $\Gamma$ distribution. The Bayesian search in MrBayes v3.1.1 with the “all genes” data set, also resulted in a sample with a mean likelihood score of $-\ln L = 93,075.814$. The mean parameter values estimated were: $A\leftrightarrow C$: 0.06422, $A\leftrightarrow G$: 0.158, $A\leftrightarrow T$: 0.175, $C\leftrightarrow G$: 0.070, $C\leftrightarrow T$: 0.512, $G\leftrightarrow T$: 0.02118 for the GTR model, estimated base composition was $A = 0.272, C = 0.217, G = 0.311, T = 0.300$, $\alpha = 0.303$ for the $\Gamma$ distribution. Bootstrap values are presented on the maximum likelihood topology Fig.S1.

Once again, the key differences present in the “all genes” data set tree relative to the complete data set tree consists of a lack of resolution for certain taxa, but not an entirely different tree, suggesting that the missing data for taxa are not contributing a disproportionate effect on the topology. The major effect produced by removing missing data, once more, was to slightly lower the bootstrap support at some of the nodes, which is expected since fewer characters are present in the “all genes” data set. In addition, it is worth noting that the 95% credibility sets of trees for each model are identical. This suggests, with respect to the phylogenetic estimate, both models are coming to the same conclusion.

The maximum likelihood analysis of the complete dataset in MrBayes v3.1.1 using the mixed model of sequence evolution resulted in a sample of trees with a mean likelihood score of $-\ln L = 118005.208$. Posterior probabilities recovered for each clade are reported in Table 1 of the paper. Values for the mixed model analysis tended to agree with those of the combine, single common model Bayesian analysis.

Figure S1. Maximum likelihood topology recovered with a GRT + $\Gamma$ model of sequence evolution with the complete data set. Values above branches represent Bayesian posterior probabilities, maximum likelihood bootstrap values, and maximum parsimony bootstrap values (bpp, ml bs, mp bs) greater than 50%. [(**) denotes 100% support for the clade in the respective analysis method; (-) denotes lack of support for the clade in the respective analysis method.]
Molecular dating.
Age estimation of clades varied across the various methods (Table S4). The Bayesian relaxed clock method consistently estimated much older date than either penalized likelihood (PL) or Yang’s a heuristic rate smoothing (AHRS) (see Table S4). MULTIDIVTIME estimated an age of 223.87 ± 17.8 for the age of the most recent common ancestor (mrca) of Formicidae, ~55 my older than any of the other estimation methods. The nature of Thorne’s Bayesian relaxed clock measure assumes that rate changes at nodes follow a log-normal distribution. Treating fossils as minimum age constraints forces divergence times further back in time because the tail end of the distribution is constrained by the minimum age barrier. The mean of the distribution is then forced further back in time from its minimum age. Studies have showed that putting both maximum and minimum age constraints on a node greatly aid in the estimation procedure. Without information about maximum ages of fossils, or reliable information on the age of the root of the ingroup, this estimation measure will give pathologically older estimates. That is exactly what we believe is going on in this case.

Ages values estimated by AHRS are more conservative than those from other programs, yielding considerably younger ages. We feel the AHRS results are probably underestimates due to fossils used as fixed calibrations of node ages for, as opposed to minimum age constraints. This can be seen in Table S4, where many of the subfamily ages are constrained to the oldest known fossil occurrence.

For these reasons, we report and discuss the ages estimated from the penalized likelihood analyses in the text. The age of the mrca of Formicidae range from 128 – 152 MYA, in range with findings from previous studies of ant divergence times (S95, S96, S97).

Table S4. Divergence time estimations ± 1.96 times the standard deviation (SD) of the bootstrapped samples. Bayesian results presented as ± 1.96 times the standard deviation posterior sample mean. 1 minimum age of fossils used as calibration points, otherwise maximum age of fossil used as calibration point. AHRS= a heuristic rate smoothing; PL= penalized likelihood.

<table>
<thead>
<tr>
<th>Clade</th>
<th>PL</th>
<th>PL1</th>
<th>AHRS</th>
<th>AHRS1</th>
<th>Bayesian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myrmicinae</td>
<td>113.96 ± 4.5</td>
<td>99.83 ± 4.2</td>
<td>52.01 ± 1.3</td>
<td>52.0 ± 1.2</td>
<td>148.30 ± 14.2</td>
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<tr>
<td>Formicinae</td>
<td>101.42 ± 3.8</td>
<td>92.00 ± 0.2</td>
<td>92.0 ± 0.0</td>
<td>90.0 ± 0.0</td>
<td>133.29 ± 26.5</td>
</tr>
<tr>
<td>Ectatomminae</td>
<td>92.28 ± 0.6</td>
<td>79.54 ± 0.9</td>
<td>79.0 ± 0.0</td>
<td>79.0 ± 0.0</td>
<td>131.66 ± 15.2</td>
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<tr>
<td>Heteroponerinae</td>
<td>91.77 ± 2.7</td>
<td>79.02 ± 3.0</td>
<td>53.52 ± 3.9</td>
<td>42.61 ± 4.0</td>
<td>132.64 ± 18.6</td>
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<tr>
<td>Dolichoderinae</td>
<td>96.58 ± 1.9</td>
<td>85.63 ± 2.2</td>
<td>79.0 ± 0.0</td>
<td>79.0 ± 0.0</td>
<td>124.11 ± 16.5</td>
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<tr>
<td>Aneuretinae</td>
<td>124.61 ± 4.8</td>
<td>107.65 ± 5.4</td>
<td>100.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
<td>165.02 ± 14.9</td>
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<td>Pseudomyrmecinae</td>
<td>59.18 ± 0.9</td>
<td>50.45 ± 1.2</td>
<td>44.1 ± 0.0</td>
<td>44.1 ± 0.0</td>
<td>85.64 ± 23.2</td>
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<td>Myrmeciinae</td>
<td>127.17 ± 2.2</td>
<td>108.29 ± 3.0</td>
<td>54.5 ± 0.5</td>
<td>54.5 ± 1.0</td>
<td>164.67 ± 18.2</td>
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<td>Aenictinae</td>
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<td>31.61 ± 1.1</td>
<td>23.68 ± 1.9</td>
<td>15.0 ± 1.2</td>
<td>48.77 ± 21.2</td>
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<tr>
<td>Doryliniae</td>
<td>12.01 ± 0.4</td>
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<td>22.61 ± 13.8</td>
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<td>Ecitoninae</td>
<td>52.14 ± 1.0</td>
<td>44.19 ± 2.0</td>
<td>32.92 ± 1.4</td>
<td>37.03 ± 1.5</td>
<td>73.29 ± 22.8</td>
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<tr>
<td>Cerapachyinae</td>
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<td>Not monophyletic</td>
<td>Not monophyletic</td>
<td>Not monophyletic</td>
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<tr>
<td>Leptanilloidinae</td>
<td>9.67 ± 0.4</td>
<td>8.01 ± 0.3</td>
<td>5.19 ± 0.1</td>
<td>5.58 ± 0.1</td>
<td>9.10 ± 6.6</td>
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<tr>
<td>Ponerinae</td>
<td>131.47 ± 5.9</td>
<td>110.70 ± 6.3</td>
<td>60.0 ± 0.0</td>
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<td>Agroecomyrmecinae</td>
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<td>108.24 ± 5.0</td>
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<td>44.1 ± 0.0</td>
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<td>Paraponerinae</td>
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<td>108.24 ± 5.0</td>
<td>44.1 ± 0.0</td>
<td>44.1 ± 0.0</td>
<td>166.13 ± 21.6</td>
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<td>Amblypoponinae</td>
<td>143.09 ± 5.2</td>
<td>113.29 ± 4.9</td>
<td>63.92 ± 1.9</td>
<td>70.03 ± 2.0</td>
<td>182.50 ± 19.6</td>
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<td>Proceratiniinae</td>
<td>131.94 ± 3.9</td>
<td>110.97 ± 3.5</td>
<td>44.1 ± 0.0</td>
<td>44.1 ± 0.0</td>
<td>161.97 ± 23.6</td>
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<td>Leptanillinae</td>
<td>122.98 ± 3.4</td>
<td>102.37 ± 4.1</td>
<td>70.97 ± 3.7</td>
<td>76.08 ± 4.1</td>
<td>181.45 ± 22.2</td>
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</tbody>
</table>
Formicoid Clade 146.98 ± 8.2 124.74 ± 6.5 116.215 ± 1.6 108.37 ± 1.5 194.86 ± 16.6
Poneroid Clade 152.39 ± 6.2 128.17 ± 5.9 74.76 ± 1.8 76.30 ± 1.6 201.14 ± 17.6
Leptanilloid Clade 122.98 ± 3.4 102.37 ± 4.1 70.97 ± 3.7 76.08 ± 4.1 181.45 ± 22.2
Formicidae 168.82 ± 7.6 140.61 ± 8.0 128.20 ± 2.4 124.62 ± 3.2 223.87 ± 17.8

Estimating Rates of Diversification.
A model of diversification characterized by two discretely different rates provided a better fit to the data than either a constant rate or a gradually changing rate, based on likelihood ratio tests and the Akaike information criterion (AIC) implemented by the program APE (Table S5). The estimated value of $\beta$ in Model B (2.78) suggest an increase in diversification rates and comparison of the two $\delta$ parameters in Model C suggest a six fold increase in diversification rate after the pre-specified breakpoint of 100 mya.

Table S5. Results of fitting three models to ant data. logL=Likelihood, $p$ = parameter. AIC = Akaike information criterion. Comparison of diversification rate models to model A performed with APE v1.4. (see text).

<table>
<thead>
<tr>
<th>Model</th>
<th>Description</th>
<th>logL</th>
<th>$X^2$</th>
<th>$P$</th>
<th>AIC</th>
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<td>A</td>
<td>Constant rate</td>
<td>$\delta = 0.013$</td>
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<td>B</td>
<td>Variable rate</td>
<td>$\beta = 2.78$</td>
<td>-2960.58</td>
<td>30637.31</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td></td>
<td>$\alpha = 0.011$</td>
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<tr>
<td>C</td>
<td>Variable rate before and after 110</td>
<td>$\delta_1 = 0.001$</td>
<td>-786.64</td>
<td>30637.31</td>
<td>&lt;0.001</td>
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<td></td>
<td>$\delta_2 = 0.006$</td>
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</table>

Additional results.
High support for the monophyly of the Formicidae was consistently recovered in all analyses (Table 1 of the paper; Fig. S1). Support for the currently recognized 20 subfamilies was maintained in all but 2 of the 19 subfamilies investigated here, the Cerapachyinae and Amblyoponinae. None of the analyses recovered the cerapachyinine genera as a monophyletic lineage. Although the amblyoponine genera grouped together, this clade lacked support. It should be noted, however, that a previous molecular study showed stronger support for the monophyly of the amblyoponine genera (S8).

Only moderate support for the subfamily Ponerinae was recovered. The monophyly of the Leptanillinae, and hence the leptanilloid clade, was always well supported (100% bpp & ml bs), and their basal position was recovered in all analyses. Although the basal position of the leptanilloid clade with respect to all other ant subfamilies was suggested in a previous molecular phylogenetic study (S8), this relationship has never been suggested by morphological studies. Many of the relationships recovered in this study differ from previous hypotheses of ant relationships (Fig. S2)

Figure S2. Phylogenetic hypotheses from the Formicidae proposed by current and previous studies. Nomenclature of groups in previous studies reflects classification systems at the time of their publication and may not correspond to the system recognized in the study.
References and Notes

S26. C. D. Bell, PORN*: a hierarchical likelihood ratio calculator for LINUX.
S64. J.K. Mortensen, S. B. Archibald, unpublished data.
### Appendix S1. List of all specimens, their taxonomic status (S54), voucher number, and location of voucher specimen. Location of voucher specimens are abbreviated as follows: Museum of Comparative Zoology (MCZ), Cambridge, MA, USA; California Academy of Sciences (CAS), San Francisco, CA, USA.

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