

Evaluating alternative hypotheses for the early evolution and diversification of ants

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Ants are the world's most diverse and ecologically dominant eusocial organisms. Resolving the phylogeny and timescale for major ant lineages is vital to understanding how they achieved this success. Morphological, molecular, and paleontological studies, however, have presented conflicting views on early ant evolution. To address these issues, we generated the largest ant molecular phylogenetic data set published to date, containing ≈6 kb of DNA sequence from 162 species representing all 20 ant subfamilies and 10 aculeate outgroup families. When these data were analyzed with and without outgroups, which are all distantly related to ants and hence long-branched, we obtained conflicting ingroup topologies for some early ant lineages. This result casts strong doubt on the existence of a poneroid clade as currently defined. We compare alternate attachments of the outgroups to the ingroup tree by using likelihood tests, and find that several alternative rootings cannot be rejected by the data. These alternatives imply fundamentally different scenarios for the early evolution of ant morphology and behavior. Our data strongly support several notable relationships within the more derived formicoid ants, including placement of the enigmatic subfamily Aenictogitoninae as sister to *Dorylus* army ants. We use the molecular data to estimate divergence times, employing a strategy distinct from previous work by incorporating the extensive fossil record of other aculeate Hymenoptera as well as that of ants. Our age estimates for the most recent common ancestor of extant ants range from ≈115 to 135 million years ago, indicating that a Jurassic origin is highly unlikely.

divergence dating | Formicidae | long-branch attraction | phylogeny

Ants (Hymenoptera:Formicidae) are the world's most successful group of eusocial insects. They constitute 15–20% of the animal biomass in tropical rainforests (1, 2) and occupy keystone positions in many terrestrial environments (3). Ants are among the leading predators of invertebrates in most ecosystems and are also prominent herbivores in many neotropical communities. Various ant species participate in symbiotic relationships with >465 plant species in >52 families (4), with thousands of arthropod species (5, 6), and with as-yet-unknown numbers of fungi and microorganisms (7). Some ant lineages have evolved astonishing adaptive specializations [agriculture of fungi, seed harvesting, herding and milking of other insects, communal nest weaving, cooperative hunting in packs, social parasitism, and slave-making (6)] that have fueled the curiosities of scientists as well as the general public.

Understanding the sequence of events contributing to the rise of ants to ecological dominance requires a robust phylogeny of their early evolution and a reliable timescale for their diversification. However, both the age of ants and the relationships among their earliest evolving lineages remain controversial. Ant fossils from the Cretaceous are relatively scarce (8, 9), although their abundance and diversity increases markedly in the Paleogene (10–12). The hymenopteran fossil record suggests that the origin of crown-group ants (i.e., the most recent common ancestor of all living ant species) occurred no earlier than 120 Mya (13). This interpretation is contradicted by several molecular divergence dating studies that consistently estimate older ages for ants (14–17). Molecular data

have also generated some surprising phylogenetic results, including the conclusion that the subfamily Leptanillinae, a group of specialized subterranean predators (18, 19), is the sister group to the rest of the ants (17, 20, 21). This conclusion contradicts all previous hypotheses about ant relationships. Thus, whereas our understanding of ant phylogeny has improved, many outstanding questions remain unresolved because of apparent conflicts between fossil, morphological, and molecular data. The current state of affairs constrains our ability to reconstruct the tempo and mode of ant evolution.

We addressed these issues by analyzing the most comprehensive molecular data set for ants published to date. We generated ≈6 kb of DNA sequence data from seven nuclear gene fragments, sampling 151 ant species spanning all 20 extant subfamilies. Using these data, we evaluated alternative hypotheses about the ages and relationships of the oldest lineages of ants. We demonstrate that a basal (i.e., sister-group) position for Leptanillinae is by no means certain. We discuss how viable alternative scenarios alter our inferences about the evolution of key ecological and behavioral traits of ants. Our divergence-dating analyses, calibrated with a combination of ant and other hymenopteran fossils, indicate that the origin of extant ants occurred sometime in the early Cretaceous ≈115–135 Mya.

Results and Discussion

Phylogenetic Relationships Among Basal Ant Lineages. Applying a range of analytical methods and strategies to our data resulted in conflicting views on the early diversification of ants. All analyses of our data generated strong support for the formicoid clade, which contains 14 of the 20 ant subfamilies (Fig. 1 and Table 1), but relationships among the remaining major lineages were more problematic. Bayesian analyses of the complete data set support, with strong posterior probability (PP) of 1.0, a poneroid clade as the sister group to the formicoids. This poneroid clade consists of the following subfamilies: Agroecomyrmecinae, Amblyoponinae, Paraponerinae, Ponerinae, and Proceratiinae. Maximum likelihood (ML) recovers this clade but with only weak bootstrap support (BS = 68). In contrast, the most parsimonious (MP) trees indicate that poneroids form a paraphyletic group (Fig. 3, which is published as supporting information on the PNAS web site), with the am-

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Abbreviations: ML, maximum likelihood; BS, bootstrap support; MP, most parsimonious; PP, posterior probability under Bayesian analysis.

Data deposition: The sequences reported in this study have been deposited in the GenBank database (accession nos. AY867421–AY867498 and EF012824–EF013787). The aligned, concatenated data matrix has been deposited in TreeBASE database, www.treebase.org (matrix accession no. M2958).

See Commentary on page 18029.

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Table 1. Support values and divergence times for major ant lineages under several analytical regimes

Node	Taxon	Estimated crown-group ages								
		Support values			Root node = 145 Mya			Root node = 185 Mya		
		PP	ML BS	MP BS	Tree A	Tree B	Tree C	Tree A	Tree B	Tree C
a	Formicidae (ants)	100/na	100/na	100/na	116 ± 3.8	117 ± 3.6	111 ± 3.4	133 ± 6.0	137 ± 6.2	127 ± 5.3
b	poneroids	100/-	68/-	-/-	100 ± 6.1	na	na	115 ± 8.2	na	na
c	formicoids	100/100	100/100	99/100	105 ± 3.5	103 ± 2.4	106 ± 3.4	119 ± 5.5	116 ± 5.0	120 ± 5.2
d	dorylomorphs	100/100	100/100	100/100	77 ± 4.9	77 ± 3.8	76 ± 5.3	88 ± 5.9	87 ± 5.2	86 ± 6.6
e	myrmeciomorphs	100/100	95/92	93/93	92 ± 4.6	91 ± 4.2	93 ± 5.7	103 ± 6.4	101 ± 6.2	103 ± 7.2
f	dolichoderomorphs	100/100	100/100	82/83	91 ± 4.4	90 ± 3.4	92 ± 4.8	100 ± 6.4	99 ± 5.4	101 ± 6.4
g	ectaheteromorphs	100/100	99/100	80/78	81 ± 6.5	82 ± 6.8	83 ± 7.2	90 ± 8.6	90 ± 9.0	92 ± 8.9
h	Leptanillinae	100/100	100/100	100/100	74 ± 8.3	76 ± 8.6	60 ± 7.3	86 ± 10.2	89 ± 10.5	68 ± 8.8
i	Ponerinae	100/100	100/100	95/96	79 ± 6.3	90 ± 6.3	86 ± 7.1	90 ± 8.1	103 ± 8.3	98 ± 8.4
j	Dolichoderinae	100/100	100/100	100/100	71 ± 3.9	71 ± 3.4	72 ± 4.1	75 ± 5.1	75 ± 4.4	76 ± 5.3
k	Formicinae	100/100	100/100	100/100	77 ± 3.5	77 ± 3.2	78 ± 3.5	82 ± 4.4	82 ± 4.3	83 ± 4.2
l	Myrmecinae	100/100	100/100	100/100	82 ± 4.3	81 ± 3.9	82 ± 4.2	89 ± 5.8	87 ± 5.4	89 ± 5.4

Node labels correspond to those used in Fig. 1. Support values are from three methods of phylogenetic analysis: PP, posterior probability under Bayesian analysis; ML BS, maximum likelihood bootstrap; MP BS, parsimony bootstrap. Under each method, the first support value is from an analysis including both the outgroups and ingroups, whereas the second value is from an analysis including ingroups only. Crown-group ages were estimated under penalized likelihood using two alternative fixed ages for the basal outgroup node (root node = 145 Mya or 185 Mya) and three alternative topologies (trees A, B, and C, which correspond to those depicted in Fig. 1, Fig. 2 rooting 1, and Fig. 2 rooting 5, respectively). Ages are in millions of years ago (Mya), and confidence limits are shown as ± 1.96 SD of 100 bootstrap replicates. na, not applicable; -, <50%.

and to the ingroup (the ants) are relatively long (Fig. 1). To address this issue, we conducted additional analyses in which the outgroup species were excluded. The trees resulting from these ingroup-only analyses provided no statistical support for a poneroid clade (Table 1). In fact, in the tree reconstructed by Bayesian analyses, poneroids cannot be monophyletic under any possible rooting. This result is supported by highly significant posterior probabilities (Fig. 2) and directly contradicts the strong Bayesian support for such a clade when outgroups are included. Removal of outgroups did not significantly affect the topology or support for other major ant lineages (Table 1).

Based on this apparent confounding effect of the outgroups, we further examined the ingroup-only topology (Fig. 2) under the assumption, suggested by other studies (24, 25), that this topology likely reflects a more accurate reconstruction of the ingroup relationships. To provide directionality to this unrooted tree, we compared a range of alternative root positions by attaching the outgroups to different branches based on *a priori* hypotheses from the literature and evaluating these alternatives within a likelihood framework. Rooting 1 corresponds to Leptanillinae as the sister group to all other ants, the prior hypothesis suggested by previous molecular work (17, 20, 21). Rooting 2 implies the monophyly of (Amblyoponinae plus Leptanillinae plus *Tatuidris*), an alternative topology consistent with evidence of shared morphological (18, 26) and behavioral features between Amblyoponinae and Leptanillinae, including adult consumption of larval hemolymph and the use of geochilomorph centipedes as prey (6, 19, 27–30). (The biology of *Tatuidris* is unknown.) Rootings 3 and 4 treat all or part of the Amblyoponinae as sister to the rest of the ants, in recognition of a recurrent theme in the literature that amblyoponines are an early branching lineage of ants (6, 31, 32). Rooting 5 preserves poneroid monophyly by including the Leptanillinae *within* the poneroids; this result also appears in several alternative analyses of our data including (i) MP analysis in which the Leptanillinae is constrained not to be the sister group of all other ants, and (ii) MP analyses of only the five protein-coding genes. Rooting 6 attaches the outgroups to a position within the formicoids, specifically to the branch that separates dorylomorphs (plus poneroids) from the remaining formicoids. This arrangement tests the notion that dorylomorphs are closely related to poneroid ants, as suggested by earlier morphological studies (26, 33, 34). Rootings 7 and 8 are similar to rooting 6, anchoring the ant tree on adjacent branches within the formicoids (Fig. 2). Finally, rooting 9 makes Myrmecinae sister to

the rest of the ants, reflecting the oft repeated idea that it represents an ancient and primitive group of ants (32, 35–37).

Under the likelihood-based Shimodaira–Hasegawa test (38), the data are significantly worse fitting when the outgroups attach to branches within the formicoids (rootings 6–9, all $P < 0.001$; see Table 2) compared with the most likely root position (rooting 1). Thus, monophyly of the formicoid group and its major constituent clades continues to be strongly upheld. However, the data are not significantly worse-fitting under all tested root positions within the poneroids (rootings 2–5; see Table 2). These results indicate that the data cannot reject several prior alternatives to the hypothesis that Leptanillinae is the sister group to all other extant ants. This indeterminacy is consistent with other studies (39–42) showing that rooting a tree with distantly related outgroups can be problematic, especially when long-branched ingroup taxa are involved, as is the case for Leptanillinae (Fig. 2).

Correct placement of the root is critical because alternative rootings imply different scenarios regarding the early evolution of ants, including the presumed phenotype of the direct ancestor to modern ants (32, 43). For example, rooting 1 suggests that early crown-group ants were specialized predators with cryptobiotic habits and reduced eyes (Fig. 2). This hypogeic ecomorph stands in contrast to the morphology of the closest stem-group fossil ants, the Sphecomyrminae (15, 44), which were large-eyed and probably generalist predators in exposed environments. Conversely, if the leptanillines are nested within the poneroids and the latter are sister to formicoids (rooting 5), the ancestral ant would be most parsimoniously reconstructed as having generalized (epigeic) habits more consonant with those of both Sphecomyrminae and the formicoids. Specialized predation and eye reduction then would be considered derived traits arising within the poneroid clade rather than part of the groundplan for ants.

Phylogenetic Relationships Within the Formicoid Ants. Our results agree with other molecular studies (15, 17, 20, 21, 45) in providing very robust support for a formicoid clade. This clade is upheld by our alternative rooting experiments described above. Within the formicoids, our analyses consistently recover the monophyly of all subfamilies except the Cerapachyinae, which is represented in our study by all five currently recognized genera (43). Lack of strong support for the monophyly of this subfamily is also evident in other morphological and molecular studies (16, 17, 46). Our analyses also provide compelling evidence (PP = 1.0, ML BS >90) for the

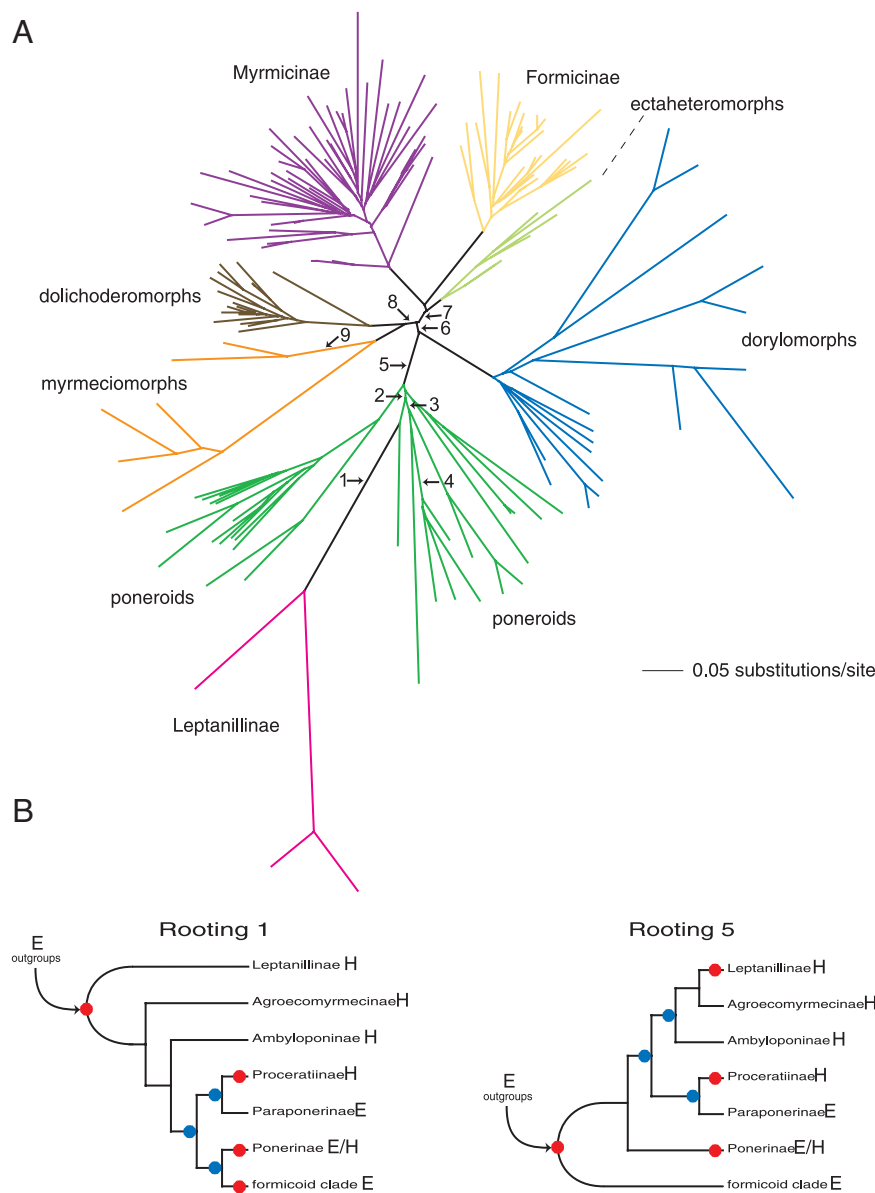


Fig. 2. Unrooted ant phylogeny with alternate attachment points of outgroups to the tree. (A) Unrooted Bayesian tree with branch lengths, obtained from analysis of ingroup-only (ant) data set, with nine possible rootings indicated by arrows. Color scheme for taxa are as in Fig. 1. (B) Schematic of relationships indicated by two of these alternate rootings. Posterior probabilities of 1.0 are indicated by red circles and of 0.95–0.99 by blue circles. The posterior probability value that applies to the bipartition at the root is placed at the midpoint of that bipartition. All depicted taxa are poneroids, except Leptanillinae and the formicoid clade. Taxa are categorized as either small-eyed and cryptic foragers (H, hypogeic) or as above-ground foragers with well developed eyes (E, epigeic). The few hypogeic taxa that occur in the formicoid clade are assumed to be secondarily derived.

following suprasubfamilial clades: dorylomorphs (army ants and relatives), myrmeciomorphs (Myrmecinae plus Pseudomyrmecinae), “dolichoderomorphs” (Aneuretinae plus Dolichoderinae), and “ectaheteromorphs” (Ectatomminae plus Heteroponerinae) (Table 1). Many relationships along the formicoid backbone have strong statistical support, with a major exception being the putative sister-group relationship between Myrmicinae and Formicinae.

The formicoid clade not only contains species-rich and highly derived taxa such as the Myrmicinae, Formicinae, and the army-ant group, but also includes several groups (Ectatomminae, Heteroponerinae, and Myrmecinae) considered to represent “primitive” ant lineages on behavioral and morphological grounds (47, 48). The interleaving of these lineages among other formicoid subfamilies indicates that the derived social traits characteristic of most formicoids, such as trophallaxis, complex chemical communication, mass recruitment, and marked queen/worker polymorphism, likely originated several times independently.

Our data provide molecular confirmation of the phylogenetic position of Aenictogitoninae. This subfamily contains a single genus, *Aenictogiton*, with seven rarely collected species. All species are known only from the male caste; females (workers and queens)

have never been discovered. These ants have long been associated with army-ant males based on overall morphological similarity (49), and a recent morphological phylogeny placed *Aenictogiton* as sister to the army ant genus *Dorylus* (46). Our molecular phylogeny sustains this position with very high support ($PP = 1.0$; $ML/MP BS \geq 97$) in all analyses.

Within the two largest ant subfamilies, Formicinae and Myrmecinae, the data reconstruct with strong support several notable relationships that have significant implications for morphological and behavioral evolution in ants. Three genera of myrmecine ants (*Myrmica*, *Manica*, and *Pogonomyrmex*) long considered “primitive” members of the subfamily on the basis of generalized morphology do in fact lie outside the “core Myrmecinae” (the remainder of the subfamily). The myrmecine seed-harvesting ant genus *Messor* is not monophyletic; instead, the New World (*Messor andrei*) and Old World (*Messor denticornis*) species arise at different locations in the tree, supporting two parallel origins of the granivore morphotype. *Camponotus*, a hyperdiverse ant genus, also consists of a polyphyletic assemblage, with the subgenus *Colobopsis* (represented in our study by *Colobopsis conithorax* and *C. BCA01*) separated from other *Camponotus* species by intervening genera

Table 2. Comparison of alternative root positions using the likelihood-based Shimodaira–Hasegawa test

Position	-lnL value	P value
Rooting 1	108793.84279	—
Rooting 2	108815.36794	0.321308
Rooting 3	108817.94590	0.301352
Rooting 4	108815.36792	0.321296
Rooting 5	108815.00662	0.361248
Rooting 6	108890.72793	0.000488
Rooting 7	108898.60098	0.000160
Rooting 8	108898.60109	0.000160
Rooting 9	108918.71129	0.000024

The nine root placements are depicted in Fig. 2A.

(*Calomyrmex*, *Polyrhachis*). Several tribes within the Formicinae (*Lasiini*, *Plagiolepidini*) and Myrmicinae (*Pheidolini*, *Solenopsidini*, *Stenammini*) seem to be nonmonophyletic with very strong support, presaging future modification of the current classification. We caution, however, that some infra-subfamilial relationships remain poorly supported and will require larger samples of taxa and genes before defensible changes can be made.

The Timescale of Ant Evolution. To estimate divergence times for ant lineages, we used a relaxed molecular clock by using the penalized likelihood method (50). We incorporated minimum age constraints on specific nodes by using fossil data from both ants (37 nodes) and other aculeate Hymenoptera (4 nodes). We also assigned a range of fixed ages to the basal outgroup node, the most recent common ancestor of all sampled Aculeata except Chrysidoidea. Our lower bound estimate for the origin of crown-group ants ranges from 111 ± 3.4 to 117 ± 3.6 Mya, depending on the topology assumed (Table 1). This estimate is based on the assignment of 145 Mya to the basal outgroup node, a defensible minimum age given the presence of both vespoid (*Scoliidae*, *Vespidae*) and apoïd (*Angarosphecidae*) aculeates in deposits ≈ 140 Mya (51–53).

Our upper bound estimate for the origin of crown-group ants spans 127 ± 5.3 to 137 ± 6.2 Mya, again depending on the topology used (Table 1). This estimate is based on using a fixed age of 185 Mya for the basal outgroup node. We consider this the oldest plausible date that could be assigned to this node for the following reasons.

- There is an extensive fossil record of Hymenoptera, with nearly all modern families and/or superfamilies represented (54).
- Major lineages of Hymenoptera appear in the same sequence in the fossil record as they are inferred to have arisen based on phylogenetic analyses of extant taxa. The first to appear is the Xyelidae 230 Mya, followed by other Symphyta 190 Mya, Apocrita 185 Mya, stem-group Aculeata 155 Mya, and crown-group Aculeata 140 Mya (13, 54).
- Jurassic hymenopteran assemblages contain a diverse array of Symphyta and nonaculeate Apocrita but no crown-group Aculeata. The Bethyloymidae, interpreted as stem-group aculeates, are known from 155–125 Mya (51, 54, 55).

Therefore, an age of 185 Mya for our basal outgroup node, which is nested *within* the crown-group aculeates, is very likely an overestimate because it implies that multiple undiscovered ghost lineages of aculeates occurred throughout the middle and late Jurassic, an unlikely scenario given the quality of the hymenopteran fossil record. By this line of reasoning, the ant divergence time estimates obtained using this calibration represent hard upper bounds.

Our estimates of divergence dates are robust to several potential sources of error from the fossil record. Exclusion of the four outgroup minimum-age calibrations resulted in identical or nearly identical age estimates. Furthermore, we tested the sensitivity of our results to different age assignments to three deposits of somewhat

uncertain age (Dominican amber, Sicilian amber, Green River). This analysis was motivated by a previous study (17) that reported a 28 million-year age difference in the lower (140 Mya) and upper (168 Mya) estimates for extant ants, with these differences based solely on alternate minimum-age calibrations for these three fossil strata. When we employed the same alternate calibrations on equivalent nodes in our data set, we saw a much smaller difference of 0–2 million years (depending on the particular topology and outgroup node age used) in the age estimate for extant ants.

The range of dates estimated for the origin of extant ants in the present study (≈ 115 to ≈ 135 Mya) contrasts with the considerably older ages (≈ 140 to ≈ 168 Mya) generated in this previous study (17). Both studies used the penalized likelihood method to infer these dates, but, because the previous study did not indicate which node(s) were assigned fixed and/or maximum ages, these discrepancies cannot be fully evaluated. Our analyses, however, cast doubt on these older estimates by showing that they are incompatible with the hymenopteran fossil record. For example, the fixed age of the outgroup node in our data set would have to be ≈ 230 million years to generate an age of 160 million years for ants. Such an ancient date would imply almost 100 million years of multiple undiscovered lineages of aculeate Hymenoptera, a result strongly inconsistent with the known preservation sequence and level of completeness of the fossil record.

In light of the full hymenopteran fossil record, we conclude that there is no need to posit a long, unrecorded history of early ants. Crown-group ants are known from deposits as old as 100 Mya (9, 56), and our molecular results indicate that they arose no more than 10–40 million years before this time. Of course, stem-group ants, such as Sphecomyrminae and Armaniidae (13, 43), must have originated earlier than this.

Recent synthesis of ecological, natural history, and evolutionary data proposes that ants diversified in concert with the angiosperms (3), with the current ecologically dominant ant groups radiating primarily in the Paleogene (3) or in the late Cretaceous (17), during times of angiosperm forest proliferation. Our analyses suggest that many ant subfamilies probably originated toward the end of the Cretaceous (Table 1; see also Table 3, which is published as supporting information on the PNAS web site), with most extant genera not evolving until the Paleogene. Given difficulties both in determining what exactly constitutes an ecologically dominant ant lineage and in dating the diversification of angiosperms (57), it is unclear at present how much these dating estimates are able to validate either version of this hypothesis. This area should be a fruitful topic for future research.

Concluding Remarks. Molecular phylogenetics has the potential to illuminate how ants evolved to become such dominant and diverse organisms in many modern ecosystems. Our analyses, however, demonstrate that caution needs to be exercised in this endeavor. Several recent analyses, including those reported here, have produced unexpected hypotheses regarding the phylogeny of ants and the timescale for their diversification. Some of these novel results, such as the existence of a formicoid clade previously unsuspected based on morphology, are well supported by the data and are robust to a range of analytical strategies. But other results remain sensitive to analytical methods and assumptions. This sensitivity seems to be the result, at least in part, of long-branch attraction between the outgroups and some ingroup taxa, although other factors such as data saturation and rapid diversification may also inhibit our ability to reconstruct these relationships. Specifically, we have shown that several alternative hypotheses for the relationships among the earliest ant lineages cannot be rejected by currently available molecular data. In addition, we show that, by taking into account the fossil record for Hymenoptera as a whole, we obtain divergence time estimates for ants that are considerably younger than those of other molecular studies, with crown-group ants originating in the early Cretaceous rather than the Jurassic. Additional data and new

analytical techniques will be required to hone the timescale for ant evolution and to determine which of the several alternative phylogenies is correct.

Materials and Methods

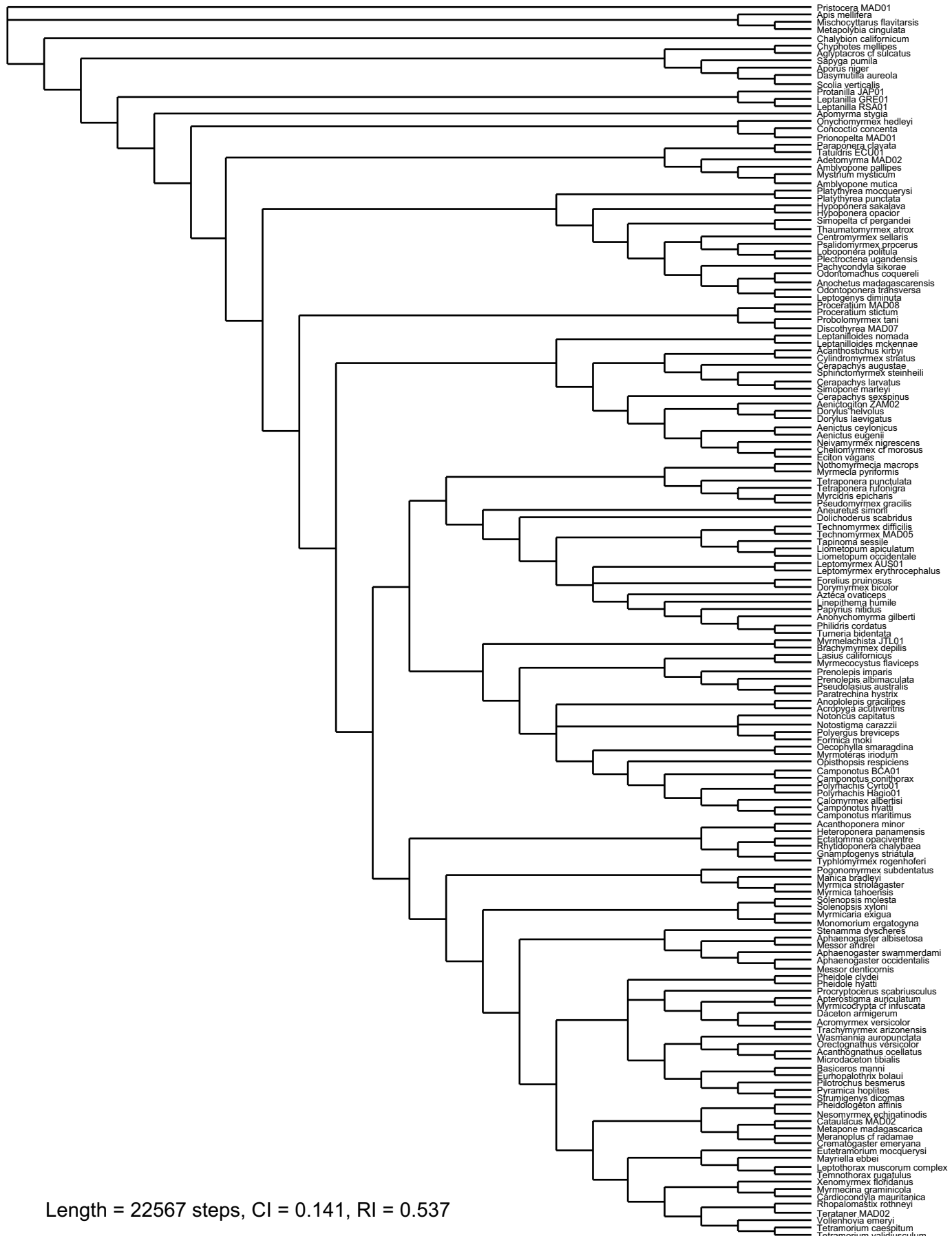
Taxon and Gene Sampling. We sampled 151 ant species, taken from all 20 extant subfamilies and from 54 of the 62 extant tribes (taxonomy follows refs. 9 and 43). For outgroups, we used 11 other aculeate wasps belonging to 10 families, including representatives from groups that have been postulated to be closely related to ants, such as Bradynobaenidae, Scoliididae, Vespidae, and Tiphiidae. Collection codes and GenBank numbers for all 162 taxa in this study are provided in Table 4, which is published as supporting information on the PNAS web site. By using conventional PCR methods (58, 59), we obtained DNA sequence data from seven nuclear genes: 1,904 aligned bp from 18S; 2,505 bp from 28S; 421 bp from wingless; 458 bp from long-wavelength rhodopsin; 639 bp from abdominal-A; 359 bp from elongation factor 1 α F1; and 517 bp from elongation factor 1 α F2. Primers for the first five genes are reported elsewhere (59). Sequence characteristics for all genes are provided in Table 5, which is published as supporting information on the PNAS web site, and primers for EF1 α F1 and EF1 α F2 are provided in Table 6, which is published as supporting information on the PNAS web site. We obtained sequence data from all taxa for all genes. The aligned, concatenated data matrix has been deposited in the TreeBase database (matrix accession no. M2958).

Phylogenetic Inference. We inferred phylogenies using MP, ML, and Bayesian methods. Nucleotide substitution models for ML and Bayesian analyses were selected by using the Akaike Information Criterion (AIC) (60). Branch support was assessed by using the nonparametric BS (61) under MP and ML, and posterior probabilities were assessed under Bayesian methods. Analyses were conducted with and without outgroups to test their effect on the ingroup topology (22–24). Alternate placements of the outgroups on the ingroup-only tree were compared by using the Shimodaira–Hasegawa test (38, 62). Detailed information on the implementation of all phylogenetic methods is found in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

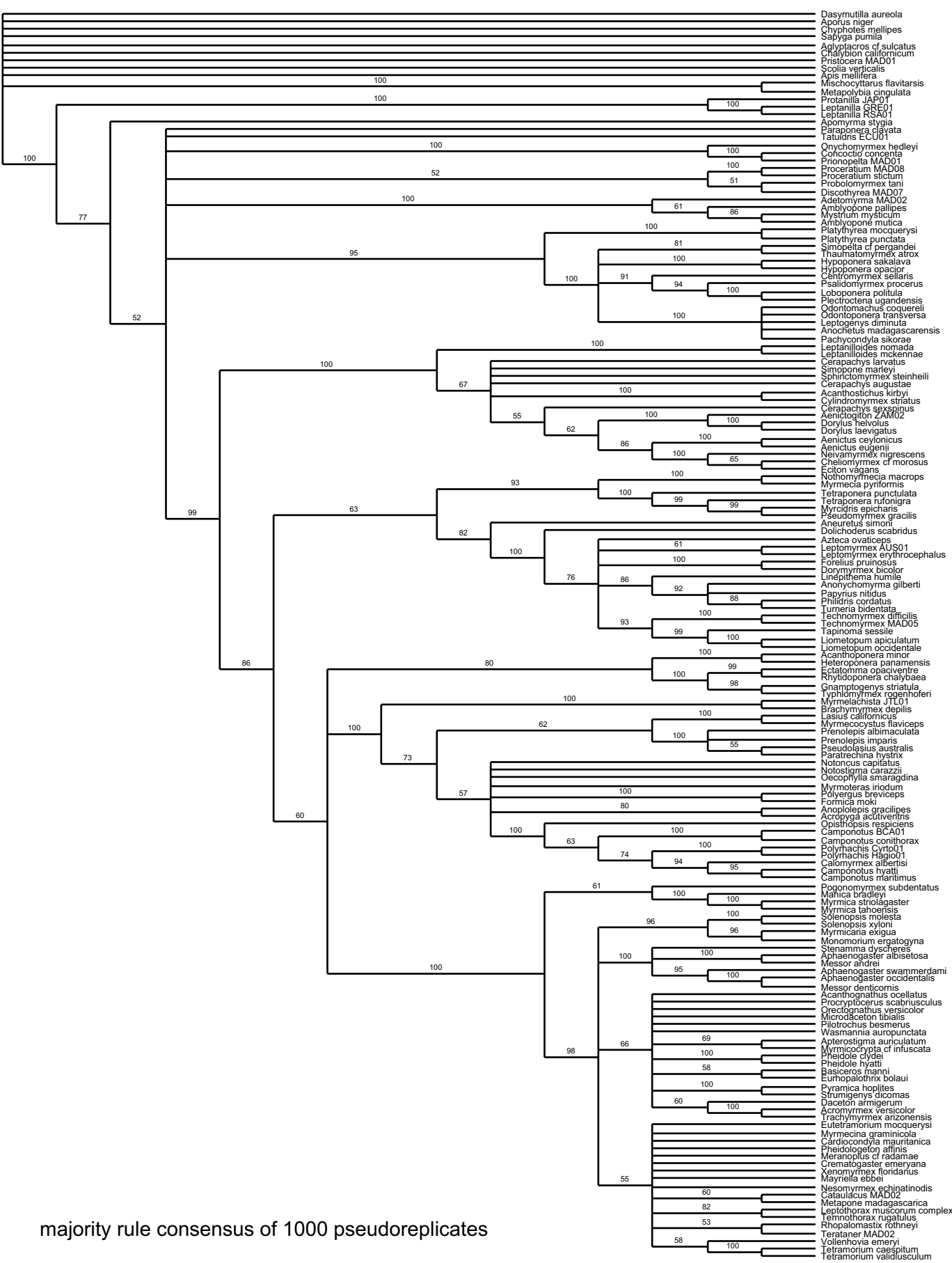
Divergence Dating. We inferred divergence dates by using the penalized likelihood approach implemented in r8s v1.7 (50, 63). We calibrated 41 nonredundant nodes with minimum-age constraints, including 37 within ants and 4 within the outgroups (Table 7, which is published as supporting information on the PNAS web site). Fossils were used to calibrate stem-group taxa (64). The r8s program requires that at least one node in the tree be either fixed or constrained with a maximum age. To establish lower and upper bounds for our divergence dates, we therefore conducted separate analyses in which the root node was fixed with an age representing either the youngest (145 Mya) or the oldest (185 Mya) reasonably possible dates for this node based on the hymenopteran fossil record (see *Results and Discussion*). Confidence intervals for all estimated dates were calculated by generating 100 nonparametric BS replicates of the data set, followed by re-estimation of branch lengths and divergence times for each replicate. We analyzed three different tree topologies to gauge the impact of alternative phylogenetic hypotheses on dating estimates. These topologies are as follows: (i) the Bayesian 50% consensus topology from analysis of the entire data set (Fig. 1); (ii) the topology obtained with rooting 1 on the ingroup-only tree (Fig. 2); and (iii) the topology obtained with rooting 5 on the ingroup-only tree (Fig. 2). Additional details on the divergence dating analyses are found in *Supporting Materials and Methods*.

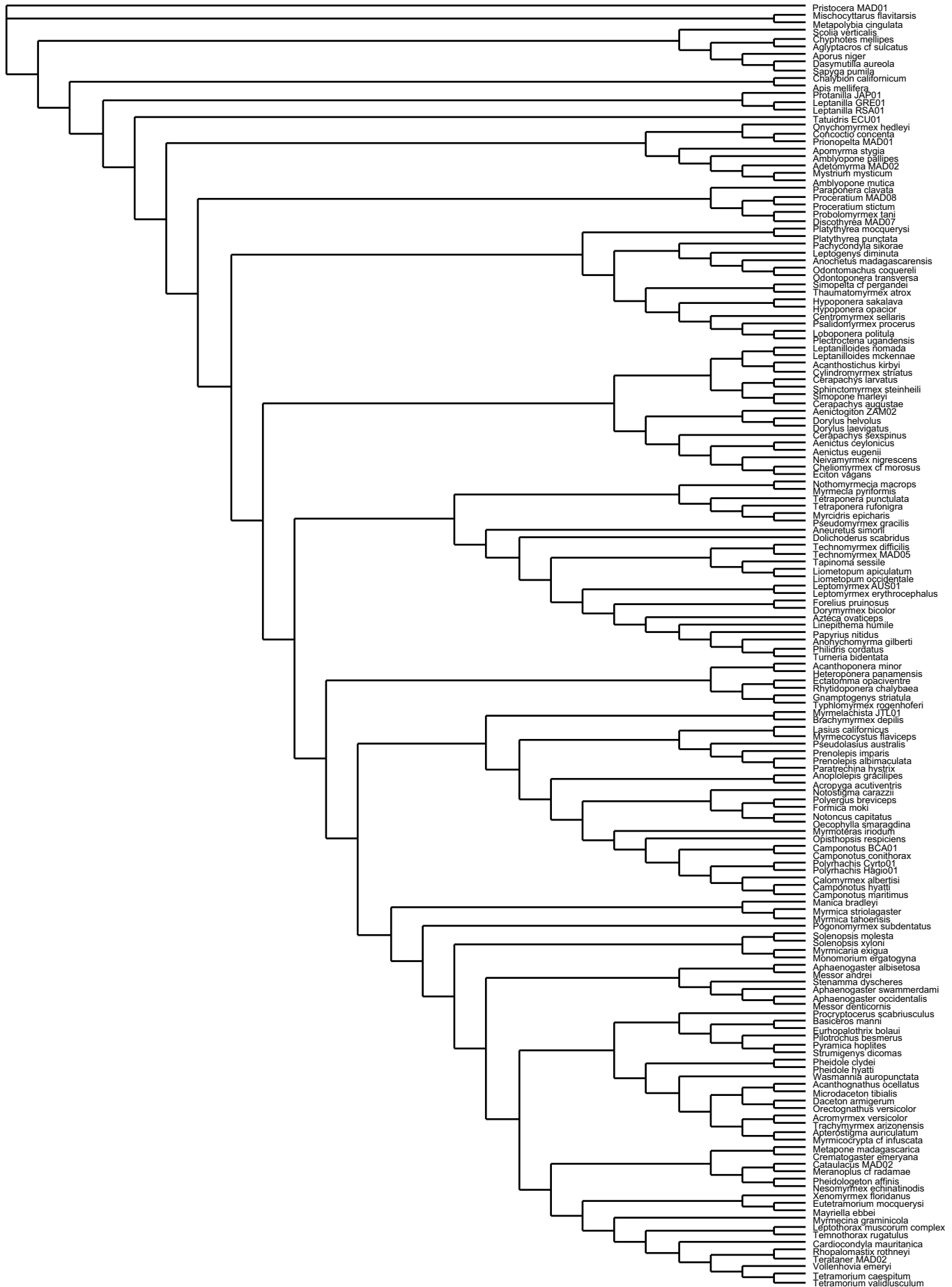
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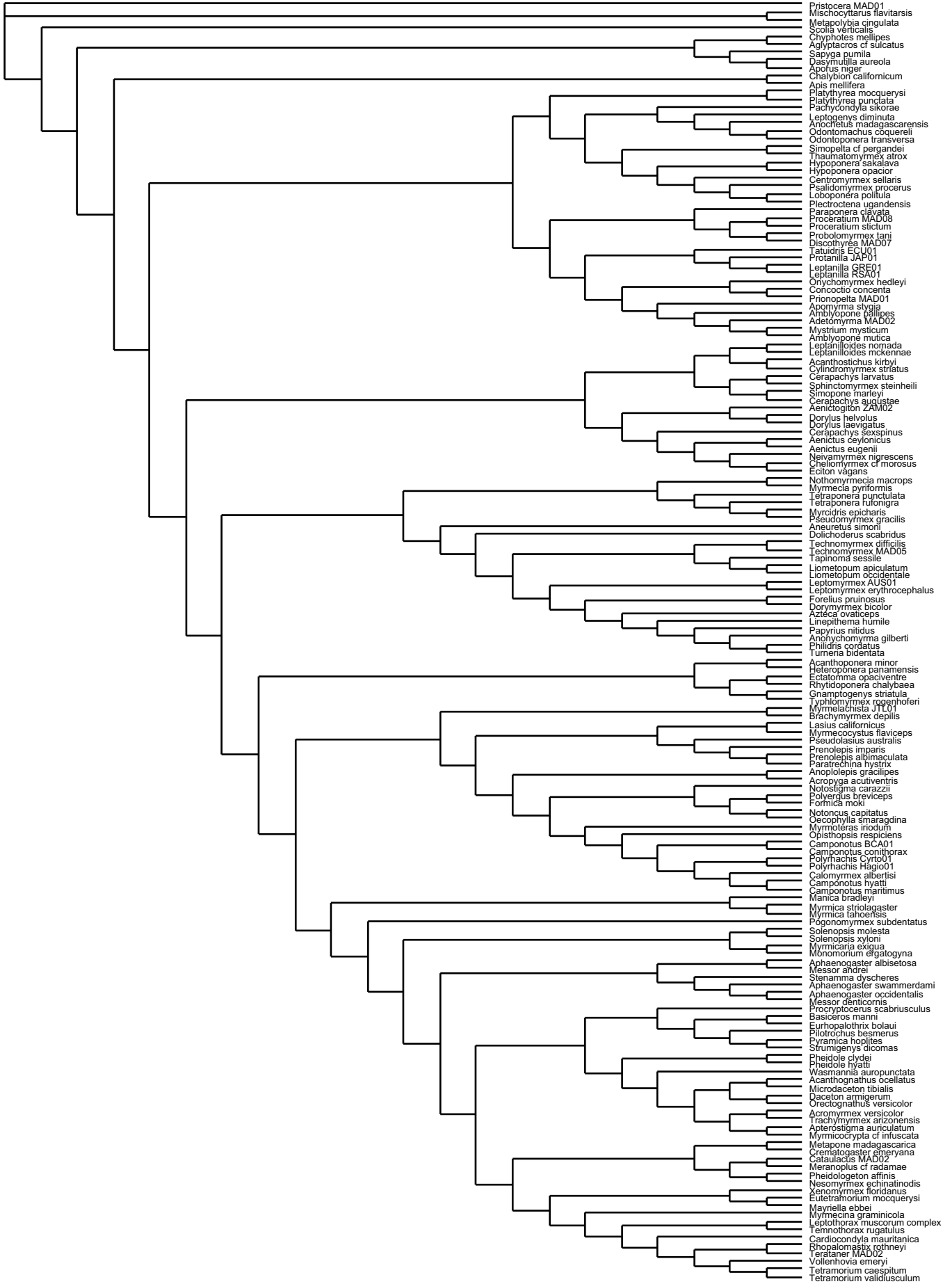
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Length = 22567 steps, CI = 0.141, RI = 0.537







- Pristocera MAD01
- Mischocyttarus flavitarsis
- Metapolybia cingulata
- Scelia verticalis
- Clypeotes mellipes
- Aglyptacros cf sulcatus
- Saigya pumila
- Dasytullia aureola
- Aporus niger
- Chalybion californicum
- Apis mellifera
- Platythyrea mocquersyi
- Platythyrea punctata
- Pachycondyla sikorae
- Leptogenys diminuta
- Anochetus madagascarensis
- Odonomachus coquereli
- Odonoponera transversa
- Simopella cf pergandei
- Thaumatomyrmex atrox
- Hypoponera sakalava
- Hypoponera opacior
- Centromyrmex sellaris
- Psalidomyrmex procerus
- Loxoponera pollula
- Plectroctena ugandensis
- Paraponera clavata
- Proceratium MAD08
- Proceratium strictum
- Probolomyrmex tani
- Discothyrea MAD07
- Jatidris ECU01
- Protanilla JAP01
- Leptanilla GRE01
- Leptanilla RSA01
- Onychomyrmex hedleyi
- Concoctio concentra
- Prionopelta MAD01
- Apomyrma sturgis
- Amblyopone baillipes
- Adetomyrma MAD02
- Myrmiarium mysticum
- Amblyopone mutica
- Leptanilloides nomada
- Leptanilloides mckennae
- Acanthostichus kirbyi
- Cylindromyrmex striatus
- Cerapachys larvatus
- Sphinctomyrmex steinhelli
- Simopone marleyi
- Cerapachys augustae
- Aenictogiton ZAM02
- Dorylus helveticus
- Dorylus laevigatus
- Cerapachys sexspinus
- Aenictus ceylonicus
- Aenictus eugeni
- Neivamyrmex nigrescens
- Cheilomyrmex cf morosus
- Ecton vagans
- Nothomyrmecia macrops
- Myrmecia pyriformis
- Tetraponera punctulata
- Tetraponera fulvigrana
- Myrcidris epicharis
- Pseudomyrmex gracilis
- Aneuretillus simoni
- Dolichoderus scabridus
- Techomyrmex difficilis
- Techomyrmex MAD05
- Tapanotis sessile
- Liomotopum apiculatum
- Liomotopum occidentale
- Leptomymex AUS01
- Leptomymex erythrocephalus
- Forelius pruinosis
- Dorymyrmex bicolor
- Zetema ovaliceps
- Linepithema humile
- Papyrius nitidus
- Anychomyrma gilberti
- Philiidris cordatus
- Turneria bidentata
- Acanthoponera minor
- Heteroponera panamensis
- Ectatomma opaciventre
- Rhytidoponera chalybaea
- Gnamptogenys striatula
- Jyphomyrmex rogenhoferi
- Myrmelachista JTL01
- Brachymyrmex depilis
- Lasius californicus
- Myrmecocystus flaviceps
- Pseudolasius australis
- Prenolepis imparis
- Prenolepis albimaculata
- Paratrechina hystrix
- Anoplolepis gracilipes
- Acropygia acutiventris
- Notostigma carazzii
- Polyergus breviceps
- Formica moki
- Notoncus capitatus
- Oecophylla smaragdina
- Myrmoteras inodum
- Clisthopis respiciens
- Camponotus BCA01
- Camponotus conthorax
- Polyrhachis GYR01
- Polyrhachis Hagi01
- Calomyrmex albertsi
- Camponotus hyatti
- Camponotus maritimus
- Manica bradleyi
- Myrmica stilogaster
- Myrmica tahosensis
- Pogonomyrmex subdentatus
- Solenopsis molesta
- Solenopsis xyloni
- Myrmica exiguus
- Monomorium ergatogyna
- Aphaenogaster albisetosus
- Messor ahirei
- Stenamma dyscheres
- Aphaenogaster swammerdami
- Messor denticornis
- Procrystocerus scabriosculus
- Basileceros manni
- Eurhopalothrix bolawi
- Pilotrochus besmerus
- Pyramica hoplites
- Strumigenys dicomas
- Pheidole elvdi
- Pheidole hyatti
- Wasmannia auropunctata
- Acanthognathus ocellatus
- Microdaceton tibialis
- Daceton armigerum
- Orectognathus versicolor
- Acromyrmex versicolor
- Trachymyrmex arizonensis
- Apterostigma auriculatum
- Myrmecocrypta cf infuscata
- Metapone madagascarcia
- Crematogaster emeryana
- Cataulacus MAD02
- Melanoplus cf radamae
- Pheidologeton affinis
- Nesomyrmex echinatoides
- Xenomyrmex floridanus
- Eutetratorium mocquersyi
- Mayriella ebbei
- Myrmecina graminicola
- Leptothorax muscorum complex
- Temnothorax rugatulus
- Cardiocondyla mauritanica
- Rhopalomyrmex rothneyi
- Terataner MAD03
- Vollenhovia emeryi
- Tetramorium caespitum
- Tetramorium validiscutum

Table 3. Expanded list of divergence time estimates for major ant lineages. Dating estimates (based on penalized likelihood) were estimated using two different fixed ages (145 mya, 185 mya) for the outgroup root node, and three alternate topologies. The outgroup root node is the most recent common ancestor of all sampled Aculeata except *Pristocera* (Bethyridae). Trees A, B, and C correspond to those depicted in Fig. 1, Fig. 2 rooting 1, and Fig. 2 rooting 5, respectively. All ages in millions of years ago (mya).

	Age of outgroup root node					
	<u>145 mya</u>			<u>185 mya</u>		
	tree A	tree B	tree C	tree A	tree B	tree C
Formicidae (ants)	116±3.8	117±3.6	111±3.4	133±6.0	137±6.2	127±5.3
poneroids	100±6.1	na	na	115±8.2	na	na
Ponerinae	79±6.3	90±6.3	86±7.1	90±8.1	103±8.3	98±8.4
Ponerini	53±4.9	59±5.4	54±5.2	61±6.4	67±6.4	62±6.2
Proceratiinae	78±9.0	85±9.1	81±9.3	90±11.0	98±11.3	91±11.0
Amblyoponinae	92±6.6	103±6.4	94±6.4	106±8.7	118±8.4	107±8.2
(<i>Mystrium</i> + <i>Amblyopone</i> + <i>Adetomyrma</i>)	42±4.9	46±5.9	43±5.4	49±5.8	53±7.1	49±6.4
(<i>Onychomyrmex</i> + <i>Concoctio</i> + <i>Prionopelta</i>)	45±6.4	48±6.8	46±6.4	51±8.0	55±8.3	51±7.8
formicoids	105±3.5	103±2.4	106±3.4	119±5.5	116±5.0	120±5.2
Leptanillinae	74±8.3	76±8.6	60±7.3	86±10.2	89±10.5	68±8.8
dorylomorphs	77±4.9	77±3.8	76±5.3	88±5.9	87±5.2	86±6.6
myrmeciomorphs	92±4.6	91±4.2	93±5.7	103±6.4	101±6.2	103±7.2
Myrmeciinae	47±5.4	46±5.2	47±5.6	52±6.5	51±6.8	52±7.3
Pseudomyrmecinae	44±1.6	44±0.4	44±0.6	47±5.2	46±3.9	46±4.4
dolichoderomorphs	91±4.4	90±3.4	92±4.8	100±6.4	99±5.4	101±6.4
Dolichoderinae	71±3.9	71±3.4	72±4.1	75±5.1	75±4.4	76±5.3
ectaheteromorphs	81±6.5	82±6.8	83±7.2	90±8.6	90±9.0	92±8.9
Ectatomminae	56±5.1	56±4.7	57±5.1	61±6.6	62±6.6	62±6.4
Heteroponerinae	39±8.1	41±8.3	42±8.1	43±9.1	45±9.4	46±8.7
Formicinae	77±3.5	77±3.2	78±3.5	82±4.4	82±4.3	83±4.2
Camponotini	44±0.1	44±0.1	44±0.3	44±0.6	44±0.9	44±1.1
Myrmicinae	82±4.3	81±3.9	82±4.2	89±5.8	87±5.4	89±5.4
core Myrmicinae	71±4.2	70±4.0	71±4.0	76±5.4	75±5.4	76±4.7

na: not applicable

Family	Subfamily	Genus	Species	Coll. Code	CASENT number	GenBank 18S	GenBank 28S	GenBank Wg	GenBank Abd-A	GenBank LW Rh	GenBank EF1aF1	GenBank EF1aF2
Formicidae	Aenictinae	Aenictus	ceylonicus	PSW 15369	CASENT0106017	EF012830	EF012958	EF013666	EF013086	EF013538	EF013215	EF013377
Formicidae	Aenictinae	Aenictus	euogenii	PSW 13926	CASENT0106069	AY867439	AY867455	AY867424	AY867411	AY867486	EF013216	EF013378
Formicidae	Aenictogitoninae	Aenictogiton	ZAM02	BLF 13532	CASENT0106126	EF012829	EF012957	EF013665	EF013085	EF013537	EF013214	EF013376
Formicidae	Agroecomyrmecinae	Tatuidius	ECU01	RA 99-208C	CASENT0423526	EF012939	EF013067	EF013775	EF013195	EF013647	EF013354	EF013516
Formicidae	Amblyoponinae	Adetomyrma	MAD02	BLF 7243	CASENT0491481	EF012828	EF012956	EF013664	EF013084	EF013536	EF013213	EF013375
Formicidae	Amblyoponinae	Amblyopona	antca	ANTC 5266	CASENT0006827	EF012832	EF012960	EF013668	EF013088	EF013540	EF013218	EF013380
Formicidae	Amblyoponinae	Amblyopone	pallipes	PSW 14753	CASENT0106070	AY703487	AY703554	AY703621	AY703688	AY703755	EF013219	EF013381
Formicidae	Amblyoponinae	Apomyrma	stygia	ANTC 1800	CASENT0007017	EF012839	EF012967	EF013675	EF013095	EF013547	EF013228	EF013390
Formicidae	Amblyoponinae	Concoctio	concenta	BLF 1742(29)	CASENT0004306	EF012855	EF012983	EF013691	EF013111	EF013563	EF013248	EF013410
Formicidae	Amblyoponinae	Mystrinum	mysticum	BLF 10131	CASENT0076622	EF012894	EF013022	EF013730	EF013150	EF013602	EF013301	EF013463
Formicidae	Amblyoponinae	Onychomyrma	hedleyi	PSW 15385	CASENT0106018	EF012901	EF013029	EF013737	EF013157	EF013609	EF013310	EF013472
Formicidae	Amblyoponinae	Prionopelta	MAD01	BLF 9001	CASENT0494610	EF012920	EF013048	EF013756	EF013176	EF013628	EF013321	EF013493
Formicidae	Aneuretinae	Aneuretus	simoni	ANTC 1752	CASENT0007014	EF012833	EF012961	EF013669	EF013089	EF013541	EF013220	EF013382
Formicidae	Cerapachyinae	Acanthostichus	kirbyi	ANTC 4000	CASENT0106071	AY867438	AY867454	AY867423	AY867470	AY867485	EF013210	EF013372
Formicidae	Cerapachyinae	Cerapachys	augustae	EMS 1160	CASENT0106072	AY867440	AY867456	AY867425	AY867472	AY867487	EF013242	EF013404
Formicidae	Cerapachyinae	Cerapachys	larvatus	PSW 13804	CASENT0106073	AY703491	AY703558	AY703625	AY703692	AY703759	EF013243	EF013405
Formicidae	Cerapachyinae	Cerapachys	sexspinus	GZ A97-2064	CASENT0104984	EF012852	EF012980	EF013688	EF013108	EF013560	EF013244	EF013406
Formicidae	Cerapachyinae	Cylindromyrmex	striatus	ANTC 4001	CASENT0106074	AY867441	AY867457	AY867426	AY867473	AY867488	EF013250	EF013412
Formicidae	Cerapachyinae	Simopone	marleyi	HGR C242	CASENT0106075	AY867446	AY867462	AY867431	AY867478	AY867493	EF013347	EF013509
Formicidae	Cerapachyinae	Sphinctomyrma	steinheili	PSW 13800	CASENT0106076	AY867447	AY867463	AY867432	AY867479	AY867494	EF013350	EF013512
Formicidae	Dolichoderinae	Anonychomyrma	gilberti	PSW 15314	CASENT0106003	EF012835	EF012963	EF013671	EF013091	EF013543	EF013222	EF013384
Formicidae	Dolichoderinae	Azteca	ovaticipes	PSW 12448	CASENT0106110	EF012842	EF012970	EF013678	EF013098	EF013550	EF013231	EF013393
Formicidae	Dolichoderinae	Dolichoderus	scabridus	PSW 13819	CASENT0106109	EF012866	EF012988	EF013696	EF013116	EF013568	EF013254	EF013416
Formicidae	Dolichoderinae	Dorymyrma	picinolor	PSW 14999	CASENT0106031	EF012862	EF012990	EF013698	EF013118	EF013570	EF013257	EF013419
Formicidae	Dolichoderinae	Forelius	brunus	PSW 14919	CASENT0106039	EF012866	EF012994	EF013702	EF013122	EF013574	EF013262	EF013424
Formicidae	Dolichoderinae	Leptomyrma	AUS01	PSW 15297	CASENT0106001	EF012873	EF013001	EF013709	EF013129	EF013581	EF013274	EF013436
Formicidae	Dolichoderinae	Leptomyrma	erythrocephalus	PSW 13808	CASENT0106077	AY703494	AY703561	AY703628	AY703695	AY703762	EF013275	EF013437
Formicidae	Dolichoderinae	Linepithema	humile	ALW 1631	CASENT0106119	EF012875	EF013003	EF013711	EF013131	EF013583	EF013277	EF013439
Formicidae	Dolichoderinae	Liometopum	apiculatum	PSW 14944	CASENT0106033	EF012876	EF013004	EF013712	EF013132	EF013584	EF013278	EF013440
Formicidae	Dolichoderinae	Liometopum	occidentale	PSW 14573	CASENT0106078	AY867449	AY867465	AY867434	AY867461	AY867486	EF013279	EF013441
Formicidae	Dolichoderinae	Papyrius	nilitus	PSW 15349	CASENT0106012	EF012905	EF013033	EF013741	EF013161	EF013613	EF013314	EF013476
Formicidae	Dolichoderinae	Philidris	cordatus	PSW 15348	CASENT0106011	EF012910	EF013038	EF013746	EF013166	EF013618	EF013320	EF013482
Formicidae	Dolichoderinae	Tapinoma	sessile	PSW 14890	CASENT0106028	EF012938	EF013066	EF013774	EF013194	EF013646	EF013353	EF013515
Formicidae	Dolichoderinae	Techomyrma	difficile	PSW 15357-2	CASENT0106111	EF012940	EF013068	EF013776	EF013196	EF013648	EF013355	EF013517
Formicidae	Dolichoderinae	Techomyrma	mandata	BLF 9872(16)	CASENT0006839	EF012941	EF013069	EF013777	EF013207	EF013649	EF013356	EF013518
Formicidae	Dolichoderinae	Turneria	biaditosa	PSW 15392	CASENT0106019	EF012948	EF013076	EF013784	EF013194	EF013656	EF013365	EF013527
Formicidae	Dorylinae	Dorylus	helvolicus	PSW 13885	CASENT0106079	AY867442	AY867458	AY867427	AY867474	AY867489	EF013255	EF013417
Formicidae	Dorylinae	Dorylus	laevigatus	ANTC 4013	CASENT0010126	EF012867	EF012989	EF013697	EF013117	EF013569	EF013256	EF013418
Formicidae	Ectoninae	Cheliomyrma	cf. morosus	ANTC 6079	CASENT0007006	EF012854	EF012982	EF013690	EF013110	EF013562	EF013246	EF013408
Formicidae	Ectoninae	Ecton	vagans	BLF 10425	CASENT0052771	EF012863	EF012991	EF013699	EF013119	EF013571	EF013258	EF013420
Formicidae	Ectoninae	Neivamyrmex	nigrescens	PSW 14695	CASENT0106080	AY867445	AY867461	AY867430	AY867477	AY867492	EF013302	EF013464
Formicidae	Ectatomminae	Ectatomma	opaciventre	SGB 336	CASENT0106081	AY703492	AY703559	AY703626	AY703693	AY703760	EF013259	EF013421
Formicidae	Ectatomminae	Gnamptogenys	striatula	PSW 15045	CASENT0106042	EF012867	EF012995	EF013703	EF013123	EF013575	EF013264	EF013426
Formicidae	Ectatomminae	Rhytidoponera	chalybaea	PSW 15295	CASENT0106000	EF012930	EF013058	EF013766	EF013186	EF013638	EF013343	EF013505
Formicidae	Ectatomminae	Typhlomyrma	rogenhoferi	RCM 111	CASENT0106082	AY703496	AY703563	AY703630	AY703697	AY703764	EF013366	EF013528
Formicidae	Formicinae	Acropyga	acuventris	PSW 15341	CASENT0106009	EF012827	EF012955	EF013663	EF013083	EF013535	EF013212	EF013374
Formicidae	Formicinae	Anoplolepis	gracilipes	PSW 15410	CASENT0106057	EF012836	EF012964	EF013672	EF013092	EF013544	EF013223	EF013385
Formicidae	Formicinae	Brachyomyrma	deplilis	PSW 14995	CASENT0106038	EF012844	EF012972	EF013680	EF013100	EF013552	EF013233	EF013395
Formicidae	Formicinae	Calomyrma	albirtisi	PSW 15325	CASENT0106006	EF012845	EF012973	EF013681	EF013101	EF013553	EF013234	EF013396
Formicidae	Formicinae	Camponotus	BCA01	PSW 15145	CASENT0106050	EF012846	EF012974	EF013682	EF013102	EF013554	EF013235	EF013397
Formicidae	Formicinae	Camponotus	conithorax	PSW 15340	CASENT0106008	EF012847	EF012975	EF013683	EF013103	EF013555	EF013236	EF013398
Formicidae	Formicinae	Camponotus	hyatti	PSW 14925	CASENT0106032	EF012848	EF012976	EF013684	EF013104	EF013556	EF013237	EF013399
Formicidae	Formicinae	Camponotus	maritimus	PSW 15202	CASENT0106083	AY867448	AY867464	AY867433	AY867480	AY867495	EF013238	EF013400
Formicidae	Formicinae	Formica	moki	PSW 14318	CASENT0106084	AY703493	AY703560	AY703627	AY703694	AY703761	EF013263	EF013425
Formicidae	Formicinae	Lasius	californicus	PSW 15164	CASENT0106045	EF012870	EF012998	EF013706	EF013126	EF013578	EF013268	EF013430
Formicidae	Formicinae	Myrmecocystus	flaviceps	PSW 15403	CASENT0106055	EF012888	EF013016	EF013724	EF013144	EF013596	EF013294	EF013456
Formicidae	Formicinae	Myrmeleachista	JTL01	PSW 15279	CASENT0106049	EF012889	EF013017	EF013725	EF013145	EF013597	EF013295	EF013457
Formicidae	Formicinae	Myrmoteras	iodium	AB 171/98	CASENT0006837	EF012893	EF013021	EF013729	EF013149	EF013601	EF013300	EF013462
Formicidae	Formicinae	Notoncus	capitatus	PSW 15363-3	CASENT0106015	EF012896	EF013024	EF013732	EF013152	EF013604	EF013305	EF013467
Formicidae	Formicinae	Notostigma	carazzii	PSW 10006-2	CASENT0106112	EF012897	EF013025	EF013733	EF013153	EF013605	EF013306	EF013468
Formicidae	Formicinae	Oecophylla	smaragdina	ALW 2557	CASENT0106113	EF012900	EF013028	EF013736	EF013156	EF013607	EF013309	EF013471
Formicidae	Formicinae	Opisthopsylla	respicens	PSW 15395-1	CASENT0106020	EF012902	EF013030	EF013738	EF013158	EF013610	EF013311	EF013473
Formicidae	Formicinae	Paratrechina	hystrix	PSW 14990	CASENT0106037	EF012906	EF013034	EF013742	EF013162	EF013614	EF013316	EF013478
Formicidae	Formicinae	Polyergus	breviceps	PSW 15231	CASENT0106048	EF012915	EF013043	EF013751	EF013171	EF013623	EF013326	EF013488
Formicidae	Formicinae	Polyrhachis	Cyrt001	PSW 15359	CASENT0106014	EF012916	EF013044	EF013752	EF013172	EF013624	EF013327	EF013489
Formicidae	Formicinae	Polyrhachis	Hagio01	PSW 15330	CASENT0106007	EF012917	EF013045	EF013753	EF013173	EF013625	EF013328	EF013490
Formicidae	Formicinae	Prelophis	alibimaculata	PSW 14427	CASENT0106052	EF012918	EF013046	EF013754	EF013174	EF013626	EF013329	EF013491
Formicidae	Formicinae	Prelophis	imparis	PSW 14955	CASENT0106035	EF012919	EF013047	EF013755	EF013175	EF013627	EF013330	EF013492
Formicidae	Formicinae	Pseudolasius	australis	PSW 15317	CASENT0106005	EF012927	EF013055	EF013763	EF013183	EF013635	EF013339	EF013501
Formicidae	Heteroponerinae	Acanthoponera	minor	AVS 2505	CASENT0039772	EF012825	EF012953	EF013661	EF013081	EF013533	EF013209	EF013371
Formicidae	Heteroponerinae	Heteroponera	panamensis	PSW 10590	CASENT0106021	EF012868	EF012996	EF013704	EF013124	EF013576	EF013265	EF013427
Formicidae	Leptanillinae	Leptanilla	GRE01	ANTC 5267	CASENT0006814	EF012871	EF012999	EF013707	EF013127	EF013579	EF013269	EF013431
Formicidae	Leptanillinae	Leptanilla	RSA01	ANTC 4002	CASENT0106085	AY867436	AY867452	AY867421	AY867483	AY867498	EF013270	EF013432
Formicidae	Leptanillinae	Protanilla	JAP01	MY 1239	CASENT0007002	EF012925	EF013053	EF013761	EF013181	EF013633	EF013337	EF013499
Formicidae	Leptanilloidinae	Leptanilloides	mckennae	ANTC 4003	CASENT0106086	AY867440	AY867460	AY867429	AY867476	AY867491	EF013271	EF013433
Formicidae	Leptanilloidinae	Leptanilloides	nomada	ALW 2146	CASENT0106087	AY867443	AY867459	AY867428	AY867475	AY867490	EF013272	EF013434
Formicidae	Myrmecinae	Myrmica	pyrifomis	SGB 406	CASENT0106088	AY703500	AY703567	AY703634	AY703701	AY703768	EF013292	EF013454
Formicidae	Myrmecinae	Nothomyrmecia	macrops	ANTC 4010	CASENT0106089	AY703501	AY703568	AY703635	AY703702	AY703769	EF013304	EF013466
Formicidae	Myrmecinae	Acanthognathus	ocellatus	ANTC 6080	CASENT0007007	EF012824	EF012952	EF013660	EF013080	EF013532	EF013208	EF013370
Formicidae	Myrmecinae	Acromyrmex	versicolor	PSW 15404	CASENT0106056	EF012826	EF012954	EF013662	EF013082	EF013534	EF013211	EF013373
Formicidae	Myrmecinae	Aphaenogaster	albisetosa	PSW 15018	CASENT0106040	EF012837	EF012965	EF013673	EF013093	EF013545	EF013224	EF013386
Formicidae	Myrmecinae	Aphaenogaster	occidentalis									

Table 4. List of sequenced taxa, with collection codes, CASENT numbers, and GenBank accession numbers.												
Family	Subfamily	Genus	Species	Coll. Code	CASENT number	GenBank 18S	GenBank 28S	GenBank Wg	GenBank Abd-A	GenBank LW Rh	GenBank EF1aF1	GenBank EF1aF2
Formicidae	Myrmicinae	Basicores	manni	BLF 10423	CASENT0052769	EF012843	EF012971	EF013679	EF013099	EF013551	EF013232	EF013394
Formicidae	Myrmicinae	Cardiocondyla	mauritanica	PSW 14593	CASENT0106115	EF012849	EF012977	EF013685	EF013059	EF013557	EF013239	EF013401
Formicidae	Myrmicinae	Catalaulax	MAD02	BLF 10344	CASENT0487553	EF012850	EF012978	EF013686	EF013106	EF013558	EF013240	EF013402
Formicidae	Myrmicinae	Crematogaster	emeryana	PSW 14954	CASENT0106034	EF012856	EF012984	EF013692	EF013112	EF013564	EF013249	EF013411
Formicidae	Myrmicinae	Daceton	armerigerum	TRS 960410-11	CASENT0010124	EF012857	EF012985	EF013693	EF013110	EF013565	EF013251	EF013413
Formicidae	Myrmicinae	Eurhopalothrix	bolau	BLF 10436	CASENT0107554	EF012864	EF012992	EF013700	EF013123	EF013572	EF013260	EF013422
Formicidae	Myrmicinae	Euletramorium	mocquersyi	BLF 9714	CASENT0077435	EF012865	EF012993	EF013701	EF013121	EF013573	EF013261	EF013423
Formicidae	Myrmicinae	Leptothorax	muscorum_complex	PSW 14909	CASENT0106029	EF012874	EF013002	EF013710	EF013130	EF013582	EF013276	EF013438
Formicidae	Myrmicinae	Manica	bradleyi	PSW 14755	CASENT0106022	EF012878	EF013006	EF013714	EF013134	EF013586	EF013281	EF013443
Formicidae	Myrmicinae	Mayniella	ebbel	PSW 13799	CASENT0106116	EF012879	EF013007	EF013715	EF013135	EF013587	EF013282	EF013444
Formicidae	Myrmicinae	Meranoplus	cf. radamae	BLF 7226	CASENT0486686	EF012880	EF013008	EF013716	EF013136	EF013588	EF013283	EF013445
Formicidae	Myrmicinae	Messor	andrei	PSW 14837	CASENT0106051	EF012881	EF013009	EF013717	EF013137	EF013589	EF013284	EF013446
Formicidae	Myrmicinae	Messor	denticornis	PSW 13962	CASENT0106118	EF012882	EF013010	EF013718	EF013138	EF013590	EF013285	EF013447
Formicidae	Myrmicinae	Metapone	madagascariica	BLF 4840	CASENT0004528	EF012884	EF013012	EF013720	EF013140	EF013592	EF013287	EF013449
Formicidae	Myrmicinae	Microdaceton	tibialis	BLF 4000(18)	CASENT0402199	EF012885	EF013013	EF013721	EF013141	EF013593	EF013288	EF013450
Formicidae	Myrmicinae	Monomorium	ergatogyna	PSW 14915	CASENT0106030	EF012886	EF013014	EF013722	EF013142	EF013594	EF013290	EF013452
Formicidae	Myrmicinae	Myrmecina	graminicola	PSW 14658	CASENT0106054	EF012887	EF013015	EF013723	EF013143	EF013595	EF013293	EF013455
Formicidae	Myrmicinae	Myrmica	striolagaster	PSW 14963	CASENT0106036	EF012890	EF013018	EF013726	EF013146	EF013598	EF013296	EF013458
Formicidae	Myrmicinae	Myrmica	tahoenis	PSW 14767	CASENT0106091	AY703499	AY703562	AY703629	AY703649	AY703763	EF013297	EF013459
Formicidae	Myrmicinae	Myrmica	xigua	BLF 4147	CASENT0403455	EF012891	EF013019	EF013727	EF013147	EF013599	EF013298	EF013460
Formicidae	Myrmicinae	Myrmicocrypta	cf. infuscata	TRS 960410-14	CASENT0010123	EF012892	EF013020	EF013728	EF013148	EF013600	EF013299	EF013461
Formicidae	Myrmicinae	Nesomyrmex	echinatoidis	PSW 15105	CASENT0106044	EF012895	EF013023	EF013731	EF013151	EF013603	EF013303	EF013465
Formicidae	Myrmicinae	Orectognathus	vesicolor	PSW 15299	CASENT0106002	EF012903	EF013031	EF013739	EF013159	EF013611	EF013312	EF013474
Formicidae	Myrmicinae	Pheidole	clydei	PSW 14991	CASENT0106117	EF012907	EF013035	EF013743	EF013163	EF013615	EF013317	EF013479
Formicidae	Myrmicinae	Pheidole	hyatti	PSW 15214	CASENT0106046	EF012908	EF013036	EF013744	EF013164	EF013616	EF013318	EF013480
Formicidae	Myrmicinae	Pheidologeton	affinis	PSW 15364	CASENT0106016	EF012909	EF013037	EF013745	EF013165	EF013617	EF013319	EF013481
Formicidae	Myrmicinae	Pilothochus	besmerus	BLF 10501(L.O.)	CASENT0047817	EF012911	EF013039	EF013747	EF013167	EF013619	EF013321	EF013483
Formicidae	Myrmicinae	Pogonomyrmex	subdentatus	PSW 14865	CASENT0106024	EF012914	EF013042	EF013750	EF013170	EF013622	EF013325	EF013487
Formicidae	Myrmicinae	Procyptocerus	scabriusculus	PSW 15064	CASENT0106043	EF012924	EF013052	EF013760	EF013180	EF013632	EF013336	EF013498
Formicidae	Myrmicinae	Pyramica	hoplites	BLF 5138	CASENT0456151	EF012928	EF013056	EF013764	EF013184	EF013636	EF013341	EF013503
Formicidae	Myrmicinae	Rhopalomastix	rothneyi	PSW 15358	CASENT0106013	EF012929	EF013057	EF013765	EF013185	EF013637	EF013342	EF013504
Formicidae	Myrmicinae	Solenopsis	molesta	PSW 14887	CASENT0106027	EF012934	EF013062	EF013770	EF013190	EF013642	EF013348	EF013510
Formicidae	Myrmicinae	Solenopsis	xylini	PSW 15020	CASENT0106041	EF012935	EF013063	EF013771	EF013191	EF013643	EF013349	EF013511
Formicidae	Myrmicinae	Stenamma	psycheres	PSW 14850	CASENT0106023	EF012936	EF013064	EF013772	EF013192	EF013644	EF013351	EF013513
Formicidae	Myrmicinae	Strumigenys	dicomas	BLF 9176	CASENT0499800	EF012937	EF013065	EF013773	EF013193	EF013645	EF013352	EF013514
Formicidae	Myrmicinae	Tetramorium	nugatulus	PSW 14868	CASENT0106025	EF012942	EF013070	EF013778	EF013198	EF013650	EF013357	EF013519
Formicidae	Myrmicinae	Terataner	MAD02	BLF 9911	CASENT0494349	EF012943	EF013071	EF013779	EF013199	EF013651	EF013358	EF013520
Formicidae	Myrmicinae	Tetramorium	caespitum	PSW 14871	CASENT0106026	EF012944	EF013072	EF013780	EF013200	EF013652	EF013359	EF013521
Formicidae	Myrmicinae	Tetramorium	validiusculum	PSW 15315	CASENT0106004	EF012945	EF013073	EF013781	EF013201	EF013653	EF013360	EF013522
Formicidae	Myrmicinae	Trachymyrmex	arizonensis	PSW 15219	CASENT0106047	EF012947	EF013075	EF013783	EF013203	EF013655	EF013364	EF013526
Formicidae	Myrmicinae	Vollenhovia	emeryi	ANTC 4012	CASENT0010125	EF012949	EF013077	EF013785	EF013205	EF013657	EF013367	EF013529
Formicidae	Myrmicinae	Wasmannia	auropunctata	PSW 14431	CASENT0106114	EF012950	EF013078	EF013786	EF013206	EF013658	EF013368	EF013530
Formicidae	Myrmicinae	Xenomyrmex	floridanus	PSW 14443	CASENT0106053	EF012951	EF013079	EF013787	EF013207	EF013659	EF013369	EF013531
Formicidae	Paraponerinae	Paraponera	clavata	SGB 517	CASENT0106092	AY703489	AY703556	AY703623	AY703690	AY703757	EF013315	EF013477
Formicidae	Ponerinae	Anochetus	madagascariensis	BLF 10029	CASENT0498593	EF012834	EF012962	EF013670	EF013090	EF013542	EF013221	EF013383
Formicidae	Ponerinae	Centromyrmex	sellaris	BLF 4130(9)	CASENT0417147	EF012851	EF012979	EF013687	EF013107	EF013559	EF013241	EF013403
Formicidae	Ponerinae	Hypoponera	opacior	PSW 14591	CASENT0106093	AY703488	AY703555	AY703622	AY703689	AY703756	EF013266	EF013428
Formicidae	Ponerinae	Hypoponera	sakalava	BLF 9790	CASENT0494141	EF012869	EF012997	EF013705	EF013225	EF013577	EF013267	EF013429
Formicidae	Ponerinae	Leptogenys	diminuta	PSW 15347	CASENT0106010	EF012872	EF013000	EF013708	EF013128	EF013580	EF013273	EF013435
Formicidae	Ponerinae	Loboponera	pollitula	BLF 2324(20)	CASENT0003095	EF012877	EF013005	EF013713	EF013133	EF013585	EF013280	EF013442
Formicidae	Ponerinae	Odontomachus	coquerelli	BLF 8730	CASENT0499525	EF012898	EF013026	EF013734	EF013154	EF013606	EF013307	EF013469
Formicidae	Ponerinae	Odontoponera	transversa	SGB 530	CASENT0010127	EF012899	EF013027	EF013735	EF013155	EF013607	EF013308	EF013470
Formicidae	Ponerinae	Pachycondyla	sikorae	BLF 8976	CASENT0487847	EF012904	EF013032	EF013740	EF013160	EF013612	EF013313	EF013475
Formicidae	Ponerinae	Platythrepa	mocquersyi	PSW 11892	CASENT0106094	AY867437	AY867453	AY867422	AY867468	AY867484	EF013322	EF013484
Formicidae	Ponerinae	Platythrepa	punctata	ANTC 5263	CASENT0006819	EF012912	EF013040	EF013748	EF013168	EF013620	EF013323	EF013485
Formicidae	Ponerinae	Plectroctena	ugandensis	BLF 2127	CASENT0003063	EF012913	EF013041	EF013749	EF013169	EF013621	EF013324	EF013486
Formicidae	Ponerinae	Psalidomyrmex	procerus	BLF 2215	CASENT0003082	EF012926	EF013054	EF013762	EF013182	EF013634	EF013338	EF013500
Formicidae	Ponerinae	Simopelta	cf. pergandei	BLF 10392	CASENT0052744	EF012933	EF013061	EF013769	EF013189	EF013641	EF013346	EF013508
Formicidae	Ponerinae	Thaumatomyrmex	atrox	JSL 021105-01-LS08	CASENT0010121	EF012946	EF013074	EF013782	EF013202	EF013654	EF013363	EF013525
Formicidae	Proceratiinae	Discothrepa	MAD07	BLF 9800(5)	CASENT0042927	EF012859	EF012987	EF013695	EF013115	EF013567	EF013253	EF013415
Formicidae	Proceratiinae	Probolomyrmex	tani	BLF 9426(L.O.)	CASENT0041507	EF012922	EF013050	EF013758	EF013178	EF013630	EF013333	EF013495
Formicidae	Proceratiinae	Proceratium	MAD08	BLF 7871	CASENT0496881	EF012923	EF013051	EF013759	EF013179	EF013631	EF013334	EF013496
Formicidae	Proceratiinae	Proceratium	stictum	PSW 10029	CASENT0106095	AY703490	AY703557	AY703624	AY703679	AY703758	EF013335	EF013497
Formicidae	Pseudomyrmecinae	Myrcidris	epicharis	PSW 9146	CASENT0106096	AY703517	AY703584	AY703651	AY703718	AY703785	EF013291	EF013453
Formicidae	Pseudomyrmecinae	Pseudomyrmex	gracilis	PSW 14184	CASENT0106097	AY703529	AY703596	AY703663	AY703730	AY703797	EF013340	EF013502
Formicidae	Pseudomyrmecinae	Tetraponera	punctulata	RS 76/1999	CASENT0106098	AY703514	AY703581	AY703648	AY703715	AY703782	EF013361	EF013523
Formicidae	Pseudomyrmecinae	Tetraponera	rufonigra	PSW 13849	CASENT0106099	AY703515	AY703582	AY703649	AY703716	AY703783	EF013362	EF013524
Outgroups												
Apidae	Apinae	Apis	mellifera	PSW 15443	CASENT0106100	AY703484	AY703551	AY703618	AY703685	AY703752	EF013227	EF013389
Bethylidae	Pristocerinae	Pristocera	MAD01	BLF 8723	CASENT0006958	EF012921	EF013049	EF013757	EF013177	EF013629	EF013332	EF013494
Bradynobaenidae	Chyphotinae	Chyphotis	mellipes	PSW 14654-2	CASENT0106101	AY703485	AY703552	AY703619	AY703686	AY703753	EF013247	EF013409
Mutillidae	Sphaerotheraphinae	Dasyptilula	aureola	PSW 15535	CASENT0106123	EF012858	EF012986	EF013694	EF013114	EF013566	EF013252	EF013414
Pomplidae	Pomplinae	Aponus	niger	ANTC 4004	CASENT0106104	EF012840	EF012968	EF013676	EF013096	EF013548	EF013229	EF013391
Sapygidae	Sapyginae	Sapyga	pumila	ANTC 4005	CASENT0106105	EF012931	EF013059	EF013767	EF013187	EF013639	EF013344	EF013506
Scoliidae	Scollinae	Scolia	verticalis	ANTC 4007	CASENT0106107	EF012932	EF013060	EF013768	EF013189	EF013640	EF013345	EF013507
Sphecidae	Sceliphrinae	Chalybion	californicum	PSW 15440	CASENT0106103	EF012853	EF012981	EF013689	EF013109	EF013561	EF013245	EF013407
Tiphiidae	Brachyctistidinae	Agylipteros	cf. sulcatus	PSW 15482-3	CASENT0106122	EF012831	EF012959	EF013687	EF013087	EF013539	EF013217	EF013379
Vespidae	Polistinae	Metapolybia	cingulata	ANTC 4006	CASENT0106106	EF012883	EF013011	EF013719	EF013139	EF013591	EF013286	EF013448
Vespidae	Polistinae	Mischocyttarus	flaviratus	PSW 15442	CASENT0106102	AY703486	AY703553	AY703620	AY703687	AY703754	EF013289	EF013451

Table 5. Sequence characteristics for each gene. None of the genes showed significant departures from base frequency homogeneity across taxa, as evaluated using PAUP*

Gene	Total sites	Variable sites	Parsimony- informative sites	G+C %
18S rDNA	1904	310	198	0.51
28S rDNA	1690	529	366	0.59
wingless	421	282	246	0.60
LW rhodopsin	458	289	271	0.50
abdominal-A	639	291	243	0.62
EF1 α -F1	359	147	139	0.59
EF1 α -F2	517	209	196	0.51

Table 6. Primer sequences new to this study, designed for the F1 and F2 copies of elongation factor 1-alpha. Primers used for 18S rDNA, 28S rDNA, wingless, LW opsin, and abdominal-A are published in ref. 1.

Primer	Sequence (5' to 3')	Position	Source
F1-1424F	GCGCCKGCGGCTCTCACCACCGAGG	<i>Apis</i> 1424-1448	This study
F1-1829R	GGAAGGCCTCGACGCACATMGG	<i>Apis</i> 1829-1808	This study
F2-557F	GAACGTGAACGTGGTATYACSAT	<i>Apis</i> 557-579	Modified from ref. 2
F2-1118R	TTACCTGAAGGGGAAGACGRAG	<i>Apis</i> 1118-1097	This study

Position numbers correspond to those of *Apis mellifera* GenBank X52884 (F1 copy) and *Apis mellifera* GenBank AF015267 (F2 copy).

References

1. Ward PS, Downie DA (2005) *Syst Entomol* 30:310-335.
2. Degnan PH, Lazarus AB, Brock CD, Wernegreen JJ (2004) *Syst Biol* 53:95-110.

Table 7. Fossil-based minimum age calibrations used for dating analyses. We used the same ages for ant fossil deposits as those cited in ref. 11. For three deposits with two age estimates (Dominican amber, Sicilian amber, Green River) we employed the minimum of the two (e.g., 15 Ma for Dominican amber). Fossils were used to assign minimum ages to the stem-group of the associated taxon (i.e., the most recent common ancestor of the taxon and its sister-group). For example, *Kyromyrma*, a fossil formicine not assignable to any tribe (9, 17), was used to provide a minimum age for the most recent common ancestor of Formicinae and Myrmicinae.

Taxon	Age (Ma)	Fossil evidence
OUTGROUPS		
<i>Apis</i> + <i>Chalybion</i>	140	Angarosphecidae in Jianshangou Bed, Yixian Formation, China (1) and in Lulworth Formation, England (2)
<i>Aglyptacros</i>	120	<i>Architiphia</i> in Santana Formation, Brazil (3, 4)
<i>Aporus</i>	100	Undescribed fossil pompilid in Myanmar amber (4)
<i>Chalybion</i>	100	Crabronidae (5, 6) in Myanmar amber (7)
INGROUP (ANTS)		
(Aneuretinae + Dolichoderinae)	100	* <i>Burmomyrma</i> in Burmese amber (8)
Formicinae	92	* <i>Kyromyrma</i> in New Jersey amber (Turonian) (9)
Dolichoderinae	60	Dolichoderinae diverse in Sakhalin amber (Paleocene); possibly in Medicine Hat amber (Campanian) (10)
<i>Tapinoma</i>	55	<i>Tapinoma</i> in Hat Creek amber (11)
Myrmeciinae	54.5	Myrmeciinae in Olst Formation, Denmark (12)
<i>Pachycondyla</i>	48.5-53.5	<i>Pachycondyla</i> in Green River Formation, USA (13)
Agroecomyrmecinae	44.1	* <i>Agroecomyrmex</i> in Baltic amber (14)
<i>Anonychomyrma</i>	44.1	<i>Anonychomyrma</i> in Baltic amber (14)
<i>Camponotus</i> (s.l.)	44.1	<i>Camponotus</i> in Baltic amber (14)
<i>Formica</i>	44.1	<i>Formica</i> in Baltic amber (14)
<i>Gnamptogenys</i>	44.1	<i>Gnamptogenys</i> in Baltic amber (15-17)
<i>Hypoponera</i>	44.1	<i>Hypoponera</i> in Baltic amber (14)
<i>Lasius</i>	44.1	<i>Lasius</i> in Baltic amber (14)
<i>Leptothorax</i>	44.1	<i>Leptothorax</i> in Baltic amber (14)
<i>Monomorium</i>	44.1	<i>Monomorium</i> in Baltic amber (14)
<i>Myrmica</i>	44.1	<i>Myrmica</i> in Baltic amber (14)

Taxon **Age (Ma)** **Fossil evidence**

<i>Nothomyrmecia</i>	44.1	* <i>Prionomyrmex</i> in Baltic amber (14, 18)
<i>Oecophylla</i>	44.1	<i>Oecophylla</i> in Baltic amber (14)
<i>Prenolepis</i> (s.l.)	44.1	<i>Prenolepis</i> in Baltic amber (14)
<i>Proceratium</i>	44.1	* <i>Bradoponera</i> in Baltic amber and member of Proceratiinae (19)
<i>Rhytidoponera</i>	44.1	<i>Rhytidoponera</i> in Baltic amber (14)
<i>Stenamma</i>	44.1	<i>Stenamma</i> in Baltic amber (14)
<i>Tetramorium</i>	44.1	<i>Tetramorium</i> in Baltic amber (14)
<i>Tetraponera</i>	44.1	<i>Tetraponera</i> in Baltic amber (14)
<i>Pheidole</i>	34	<i>Pheidole</i> known from Oligocene (Colorado) (20)
<i>Crematogaster</i>	28.4-33.9	<i>Crematogaster</i> in Sicilian amber (21)
<i>Acropyga</i>	15-20	<i>Acropyga</i> in Dominican amber (22)
<i>Apterostigma</i>	15-20	<i>Apterostigma</i> in Dominican amber (Schultz, unpubl.)
<i>Cylindromyrmex</i>	15-20	<i>Cylindromyrmex</i> (and <i>Acanthostichus</i>) in Dominican amber (23-25)
<i>Discothyrea</i>	15-20	<i>Discothyrea</i> in Mexican amber (26), considered to be approximately the same age as Dominican amber (27).
<i>Neivamyrmex</i>	15-20	<i>Neivamyrmex</i> in Dominican amber (28)
<i>Odontomachus</i>	15-20	<i>Odontomachus</i> in Dominican amber (29)
<i>Paratrechina</i>	15-20	<i>Paratrechina</i> in Dominican amber (29)
<i>Prionopelta</i>	15-20	<i>Prionopelta</i> in Dominican amber (29)
<i>Pseudomyrmex</i>	15-20	<i>Pseudomyrmex</i> in Dominican amber (30)
<i>Pyramica</i>	15-20	<i>Pyramica</i> in Dominican amber (31)
<i>Trachymyrmex</i>	15-20	<i>Trachymyrmex</i> in Dominican amber (29)

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Charting uncertainty about ant origins

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Over a wide range of environments, up to five ant species forage every square meter of ground (1). In Amazonian rainforests, the biomass of ants dwarfs that of vertebrates (2), and in many rainforest trees, ants make up a large fraction of individual insects (3). This ecological dominance and the complexity of their societies makes their phylogeny of great interest as a glimpse into the development of the modern world in terms of the relationships between the various groups of ants, how their characteristics evolved, and when they originated. This year we have seen not one but two blockbuster articles examining ant phylogeny and time of origin of the group, one of which is by Brady *et al.* (4) in this issue of PNAS. The two articles (4, 5) agree in several important respects but disagree in others.

Early thought on ant phylogeny was bedeviled by the belief that all or most of the genera with armored cuticles and strong stings belonged in a single subfamily, the Ponerinae (6). Brown (7) pointed the way forward by suggesting that various other ant subfamilies arose within the ponerines, which are thus paraphyletic; presciently, he proposed a close relationship between the Ectatomminae (then a ponerine tribe) and the giant subfamily Myrmicinae [$>4,500$ species (8)]. However, he made no nomenclatural change, and subsequent authors tended to treat the ponerines as a single group. This tendency to agglomerate seriously compromised the ability to make sense of ant phylogeny, and for decades the procession of phylogenetic schemes was notable in its diversity rather than its stability. The crucial breakthrough came from Bolton (24), who erected a host of new subfamilies and subdivided the original subfamily Ponerinae into six; although he still placed all of these together, this recognition of difference liberated phylogeneticists to make new findings (4, 5, 9).

Bolton's (24) reorganization of ant systematics joined with the increasing ease of obtaining DNA sequence, a moderately good fossil record, and the rise of phylogenetic methods able to handle large data sets and estimate divergence dates. The first major and convincing effort to elucidate ant phylogeny at a grand scale and set it in temporal context was that of Moreau *et al.* (5) earlier this year. The study by Brady *et al.* (4) is even larger, dealing with 162

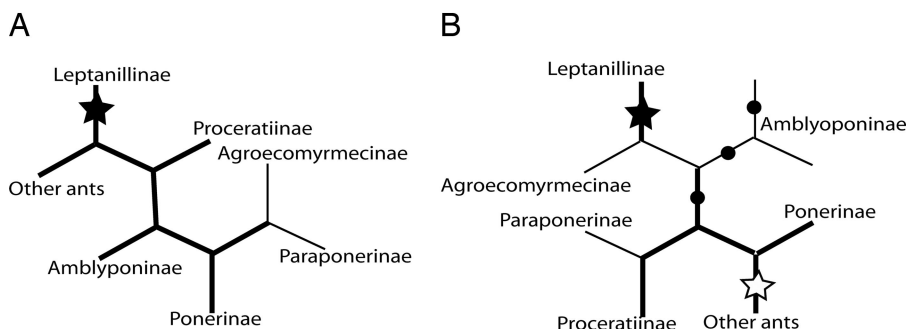


Fig. 1. Certainty becomes managed uncertainty. Analyzing the complete sequence ant data set together with outgroups yielded tree A, with the outgroups joining at the filled star, implying that the Leptanillinae are the sister group to all other ants. Analyzing just the ant sequences led to a significantly different result, tree B. Testing nine hypotheses (dots or stars) for rooting the ant tree eliminated four but left five as statistically not separable. The most likely of these, shown with a filled star, remains on the branch to the Leptanillinae, but the second most likely, shown with an open star, falls on the branch to the rest of the ants, implying that the leptanillines are closely related to the Amblyoponinae, with which they share some striking characteristics. Thick lines denote branches with posterior probability of at least 0.95. The instability of the ingroup according to whether outgroups are included in the analysis may have resulted from long branch attraction.

species from all 20 currently recognized ant subfamilies and 10 outgroups and using 6 kb of DNA sequence from seven nuclear genes. There is much agreement between the two studies. In particular, most subfamilies are monophyletic, and the two trees place them in similar positions. Brown's suggestion of a strong relationship between the Ectatomminae and the Myrmicinae is not contradicted statistically by the new findings.

There is thus now the emergence of the promise of stability in ant phylogeny, with these studies having very similar trees, but this result includes a puzzling anomaly, namely the placement of the Leptanillinae as the sister group to all other ants. Those leptanillines that have been studied are tiny, eyeless subterranean ants with an army-ant lifestyle, preying on geophilomorph centipedes like wolves on elk (10). Their bizarre habit of the queen feeding on hemolymph from her larvae also occurs in the Amblyoponinae (11), and this and morphological similarities raised suspicions that these groups are closely related. Having the Leptanillinae placed at the base of the tree of all ants (4, 5) is therefore very odd. For one thing, eyeballing the resulting tree gives the impression that the ancestral ant was eyeless and lived underground, so that the great majority of ants today must have secondarily regained eyes and moved to hunt in the open air.

Long branch attraction (12), in which groups at the ends of long branches are wrongly placed together during phylo-

geny inference, can also lead to spurious rearrangement of the ingroup taxa (13). The problem is mainly one for parsimony and will not occur for maximum likelihood or Bayesian analysis when the substitution model has been correctly specified (14), but the models now available may not reflect reality sufficiently well to avoid it in some small, but unknown, number of cases (15). To paraphrase Li (16), substitution models are naturally artificial despite the attempt to be artificially natural. Brady *et al.* (4) surmised that long branch attraction might have affected the placement of the ant groups and thus repeated the analysis with the outgroups omitted. Significant differences appeared between the two analyses (Fig. 1). In particular, the poneroids, a group of morphologically similar subfamilies, which had formed a monophyletic assemblage in the rooted tree, no longer did so when the outgroups were omitted. Next, Brady *et al.* tested nine hypotheses for the rooting of the ant tree by constraining each such link in turn and found that only four of these were eliminated statistically. The most likely one of the remaining five still placed the Leptanillinae as the sister group to the rest of

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antdom, but the second most likely of them nests them near the Amblyoponinae and allows the interpretation of the ancestral ant as an above-ground forager with eyes. Thus, an ant tree in accordance with morphological expectation is among those fitting the molecular data, but it is not a done deal.

Given the thoroughness of the phylogenetic analysis, it is striking that this sophistication did not extend to comparative analysis, for which no details have been given. Powerful Bayesian methods are now available, which, unlike earlier approaches, take uncertainty in the phylogeny into account (17, 18). Given the wealth of morphological, ecological, social, and behavioral characteristics ants present, the data now available promise a further revolution in understanding all features of ant biology.

But when did ants arise? Most modern ants (and many predatory wasps) have adults subsisting mostly on floral nectar or hemipteran exudates while hunting prey (or carrion) for the young. These characteristics speak for an association with angiosperms, which has been suggested as important to the origin of ants (19). Stemming from winged stinging ancestors, they have reduced the winged stage to a dispersal phase and adapted to life on or in the ground by females casting off their wings once they have mated. Speculating a little, dispersing, mating, and settling on the ground predisposed such insects to form small family groups, leading naturally to a strong influence of kin selection fostering the further transition to the differentiation between queens and workers (20, 21). Losing wings for foraging not only opened up the ground and its

surface, it also opened the night; many ants forage at night, but exceedingly few flying social insects manage this (22).

It is in dating the origin of ants that the emphases of this year's blockbuster ant articles differ most sharply. Moreau *et al.* (5) explored a number of dating techniques, but both Moreau *et al.* and Brady *et al.* (4) settled on the method of penalized likelihood (23) and used the same set of ant fossil ages. Whereas Moreau *et al.* appear to have used only the minimum ages of ant fossils in their analysis, Brady *et al.* also assigned two different fixed ages (145 and 185 Mya) to the most basal node, basically marking the origin of the

There is now the emergence of the promise of stability in ant phylogeny.

aculeate Hymenoptera. In each article, a maximum and minimum age was estimated for the ants. As did some earlier analyses (including one by myself and some by members of the Brady *et al.* team), Moreau *et al.* concluded that a Jurassic age for the ants is plausible; the confidence limits for these estimates range from 132.6 to 176.4 Mya. Brady *et al.* used their three "best" trees for dating, the tree resulting from analyzing all of their sequences in a single analysis (Fig. 1A) and the trees with the highest likelihood resulting from adding the outgroups to various points on the tree analyzed for ants alone (starred in Fig. 1B). The most diver-

gent of these dates and their confidence limits yields the range 105.6–143.2 Mya. The two ranges overlap, but whereas that of the earlier study overlaps the Jurassic, that of Brady *et al.* (4) does not. However, Brady *et al.* regard the older ages as problematic and hence stress a mid-Cretaceous age for the most recent common ancestor for ants. Even so, Brady *et al.* concede that there may have been ants on Earth even earlier, in the form of the apparent ant ancestors, the enigmatic fossil-only Sphecomyrminae. Where this uncertainty leaves the association with the angiosperms is a little unclear; it seems less problematic to have ants diversifying in the presence of rich angiosperm forests than before these arose, but homopterans would have provided the sugary secretions consumed by adults in preangiosperm times.

A rich interplay between systematics, morphology, and molecular phylogeny can be traced. As noted above, the systematic decision (24), based on morphology, to disaggregate the various groups then classified as tribes within the Ponerinae freed molecular phylogeneticists trying to relate the subfamilies, making possible the studies that have appeared. Molecular phylogenetic results, on the other hand, so closely linked one of Bolton's new subfamilies, the Apomyrminae, with one of the others, the Amblyponinae (9), that systematists merged the two (25). The findings include some of molecular evolutionary interest (why did the Leptanillinae evolve so much faster than other ants?), but this work has the most interest to those fascinated by the biology and evolution of this socially sophisticated and ecologically dominant group.

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