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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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Fig. 1. Bayesian tree with branch lengths, obtained from analysis of entire data set. Tree is rooted with *Pristocera* (Bethylidae). Posterior probabilities of 1.0 are indicated by red circles and of 0.95–0.99 by blue circles. The rounded rectangle encompasses basal portions of the ant tree where relationships are likely distorted as a result of rooting to distant, long-branched outgroups (see *Results and Discussion*). Lowercase letters at selected nodes refer to taxa in Table 1. Ch., *Cheliomyrmex*.

blyoponines comprising three successive basally diverging lineages, although without strong BS support (BS <0.50).

Distantly related outgroups have the potential to cause error in

the reconstruction of ingroup relationships because of long-branch attraction (22–24). This artifact may be a problem in our data set, because the branches connecting the outgroup taxa to one another

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

| | | Support values | | | Estimated crown-group ages | | | | | |
| | | | | | Root node = 145 Mya | | | Root node = 185 Mya | | |
Node	Taxon	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	Myrmicinae	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 Myrmeciinae 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 (Myrmeciinae 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 Myrmeciinae) 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 Myrmicinae, the data reconstruct with strong support several notable relationships that have significant implications for morphological and behavioral evolution in ants. Three genera of myrmicine 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 Myrmicinae" (the remainder of the subfamily). The myrmicine 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 apoid (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 Bethylonymidae, 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, Scoliidae, 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 1α F1; and 517 bp from elongation factor 1α 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 EF1αF1 and EF1αF2 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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