Newly discovered sister lineage sheds light on early ant evolution

Christian Rabeling[†‡§], Jeremy M. Brown[†¶], and Manfred Verhaagh[‡]

[†]Section of Integrative Biology, and [¶]Center for Computational Biology and Bioinformatics, University of Texas, 1 University Station C0930, Austin, TX 78712; and [‡]Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstr. 13, D-76133 Karlsruhe, Germany

Ants are the world's most conspicuous and important eusocial insects and their diversity, abundance, and extreme behavioral specializations make them a model system for several disciplines within the biological sciences. Here, we report the discovery of a new ant that appears to represent the sister lineage to all extant ants (Hymenoptera: Formicidae). The phylogenetic position of this cryptic predator from the soils of the Amazon rainforest was inferred from several nuclear genes, sequenced from a single leg. *Martialis heureka* (gen. et sp. nov.) also constitutes the sole representative of a new, morphologically distinct subfamily of ants, the Martialinae (subfam. nov.). Our analyses have reduced the likelihood of long-branch attraction artifacts that have troubled previous phylogenetic studies of early-diverging ants and therefore solidify the emerging view that the most basal extant ant lineages are cryptic, hypogaeic foragers. On the basis of morphological and phylogenetic evidence we suggest that these specialized subterranean predators are the sole surviving representatives of a highly divergent lineage that arose near the dawn of ant diversification and have persisted in ecologically stable environments like tropical soils over great spans of time.

biodiversity | Formicidae | long-branch attraction | phylogeny | soil biology

EVOLUTION

Since the Cretaceous, ants have evolved to become one of the most diverse and abundant animal families to ever appear on Earth (1–5). A robust phylogeny is indispensable for elucidating the evolutionary origin of ants and for exploring the selective forces that have produced their extraordinary specializations. Previously published studies, however, led to contradicting views of early ant evolution, in part because of high levels of morphological convergence, the secondary loss of characters, and a lack of informative paleontological data (5–14). As a result, numerous taxa have been proposed as the most basal lineage (15–23). Two recent, comprehensive molecular studies have reconstructed the formicid phylogeny, resulting in largely congruent topologies that agree that ants as a family and most of the subfamilies are monophyletic (20, 21). In contrast with previous morphological studies (5–14), the blind, subterranean subfamily Leptanillinae was recovered as sister to all extant ants, as suggested by two earlier partial molecular studies (22, 23), implying an early origin and diversification of cryptic, blind species with hypogaeic foraging habits.

Results and Discussion

Here we describe a new ant species, *Martialis heureka* (Figs. 1 and 2), comprising a new subfamily, the Martialinae, and discuss the implications of this discovery for the early evolution of ants. Morphological results confirm that *M. heureka* is clearly a member of the family Formicidae because of the presence of the metapleural gland orifice, geniculate antennae, and a morphologically differentiated petiole (Figs. 1 and 2). However, it cannot be placed within any extant or extinct subfamily (20–22, 24). *M. heureka* is close to, but not within, the subfamily Leptanillinae, because it exhibits several autapomorphies (see diagnosis below) and retains the plesiomorphic condition for other mor-

Fig. 1. Holotype worker of *Martialis heureka* gen. et sp. nov. The single specimen has been collected in the leaf litter of a terra firme rainforest near Manaus, Amazonas, Brazil. *Martialis heureka* is inferred to be the sister lineage to all extant ants. (*A*) Lateral and (*B*) dorsal view of the worker. (Scale bar: 1 mm.) Photographs courtesy of C. Rabeling and M. Verhaagh.

phological characters, like the flexible promesonotal suture and the exposed antennal sockets. Hence, we place *M. heureka* in its own subfamily, the Martialinae.

Taxonomic Treatment

Family Formicidae Latreille, 1809 (25)
Subfamily **Martialinae** Rabeling & Verhaagh, subfam. nov.
Martialis heureka Rabeling & Verhaagh, gen. et sp. nov.

Martialinae Rabeling & Verhaagh, **subfam. nov.**
Worker diagnosis: small, blind, pale, presumably subterranean ants with the following combination of characters. Putative
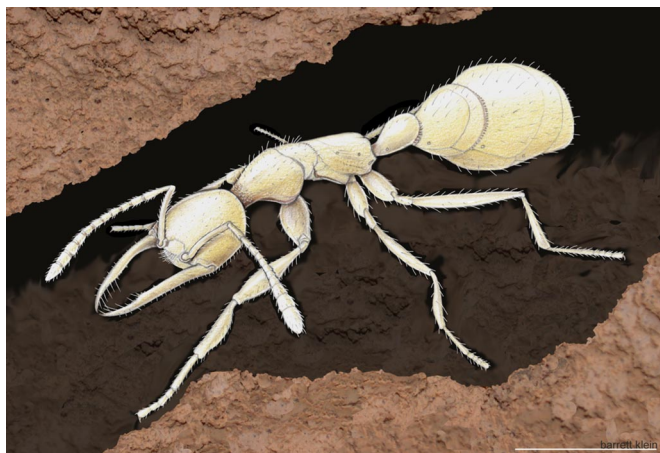
Fig. 2. Worker of *Martialis heureka* foraging in a subterranean tunnel. This drawing depicts *M. heureka* in its assumed natural habitat as inferred from its external morphology. Please see the *Inferred Biology* section for a more detailed discussion of the species' biology. (Scale bar: 1 mm.) Color pencil drawing by Barrett A. Klein.

apomorphic conditions are marked by an asterisk. (1) Prementum partly visible with closed mouthparts, not entirely concealed behind the broad labrum and stipites; (2*) clypeus highly reduced; (3*) eyes absent; (4) frontal lobes absent; (5*) antennal sockets fully exposed and distant from each other, positioned at anterior margin of clypeus and projecting anteriorly beyond it; (6*) toruli raised vertically, forming cups to hold condylar bulbs; (7) antennae 12 segmented; (8) promesonotal suture present and flexible; (9) propodeal lobes absent; (10) metapleural gland orifice slit shaped, dorsal orifice margin projecting slightly forward, but not overhanging or concealing opening; (11*) metacoxal cavity closed, with a complete cuticular annulus surrounding the cavity; (12) metasternal process absent; (13*) petiole tergosternally fused, without a trace of a suture between tergite and sternite; (14*) abdominal segment III broadly attached to segment IV but distinctly smaller than the latter, tergosternally fused; (15) helcial tergite well developed, posterior part with a girdling constriction; (16) abdominal segment IV not tergosternally fused, posttergite broadly overlapping abdominal segment V; (17) stridulitrum on presclerite of abdominal segment IV absent; (18) spiracles of abdominal segments I–IV exposed, of V–VII concealed by preceding postsclerites; (19) pygidium (tergite of abdominal segment VII) well developed, simple, neither armed with teeth or setae nor heavily sclerotized nor otherwise modified; (20) sting present; (21) metatibial gland absent; (22) tarsal claws simple, without preapical teeth.

Queen, male, and larva are unknown.

Martialis Rabeling & Verhaagh, **gen. nov.**

Type and only known species: *Martialis heureka* Rabeling & Verhaagh, sp. nov. (described below).

Worker diagnosis: including the characteristics of the subfamily description given above. (1*) Mandibles elongate, slender, forceps-like, not crossing at rest (although doing so in dried condition), without differentiated basal and masticatory margins; (2) labrum broad with a U-like insertion at apical margin; (3*) clypeus reduced to a narrow strip at the sides, with a small trapezoidal median portion between antennal sockets, densely covered with a brush of setae; (4) metanotal groove distinct; (5) propodeum evenly rounded at the transition from basal (= dorsal) to declivitous face; (6) propodeal spiracle orifice not enlarged and nearly equally distant from basal and declivitous face of propodeum and the anterior part of metapleural opening, with circular orifice directed posteroventrally; (7) petiole with short peduncle, tergosternally fused, without a trace of a suture

between tergite and sternite; (8*) presclerite of abdominal segment IV well developed, posterior part with a girdling constriction, forming the posterior constriction of a weakly defined postpetiole; (9) sting present, but small; (10) mesotibial spur present, simple; (11) metatibial spur present, shortly pectinate.
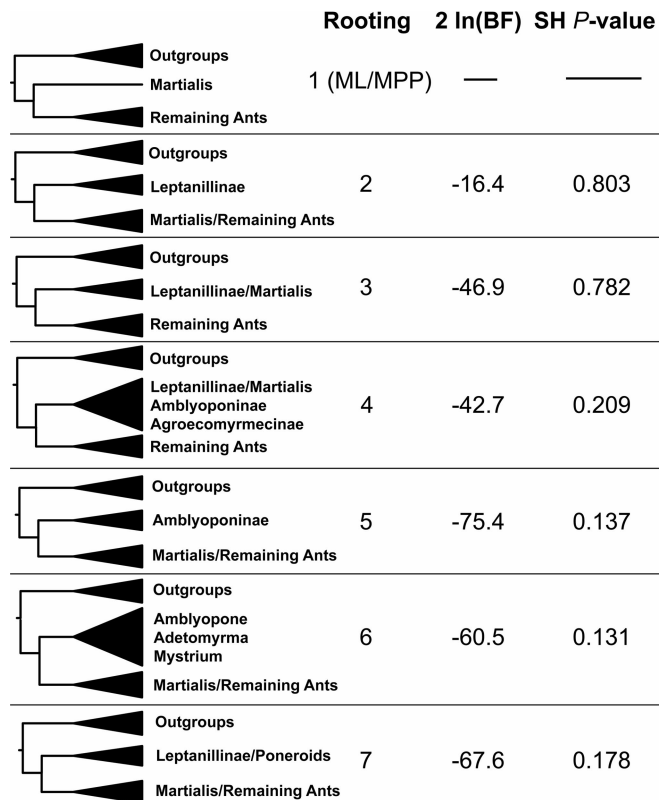
Martialis heureka Rabeling & Verhaagh, **sp. nov.**

Worker description (holotype and only known specimen). Measurements: HW 0.65 mm, HL 0.62 mm, SL 0.46 mm, FL 1.03 mm, ML 0.90 mm, WL 1.02 mm, PW 0.40 mm, PEW 0.19 mm, PEL 0.27 mm, PPW 0.34 mm, PPL 0.30 mm, HFL 0.60 mm, HTL 0.57 mm, CI 105, MI 145, SI 72, DI 45 (See Table S1 for definitions and abbreviations of morphometrics). Including the characters of the subfamily and genus description given above: small (HW 0.65, WL 1.02), pale yellow, and blind, integument in dried condition partly translucent. Very long, slender, forceps-like mandibles (ML 0.90, MI 145) inserted on outer anterior margin of head capsule, projecting straight forward to ≈5/7 of its length, then curving slightly mesally. Inner margin bearing a tiny sudmedian tooth at ≈2/5 of its length, measured from the base and a group of 3 subapical teeth at ≈5/7 of its length: a median larger tooth and a somewhat smaller tooth to each side of it. The subapical teeth and the sharply pointed mandibular tip frame an oval space. Mandibles crossed in dried condition (Fig. 1), not crossing in live individual (Fig. 2) or while stored in 80% EtOH. Head capsule bulbous in lateral view, narrowing toward posterior margin. Clypeus reduced, narrow strip between lateral head margin and wider between antennal sockets, covered with at least 20 setae that project forward, beyond the margin of antennal sockets, resembling a brush. Antennal scape relatively short (SI 72, DI 45), 1st and 2nd funicular segment ¾ and ¼ longer than 3rd funicular segment. Funiculus more than twice as long as scape. Mesosoma long and slender. Promesonotal suture present, pronotum and mesonotum presumably capable of movement relative to each other. Pronotum forming a slender cervix. Front legs enlarged: procoxae twice as long and wide as meso- and metacoxae; profemura and tibiae also enlarged. Petiole subsessile with a short anterior peduncle; petiolar node rounded, smooth, with a short sloping posterior face; no teeth or projection ventrally. Metasoma (abdominal segment IV–VIII visible) laterally compressed, drop shaped in lateral view. Head, pronotum, and legs densely covered with erect to suberect hairs and sparsely with longer erect setae; mandibular pubescence dense, consisting of short suberect hairs. Inner margin with two rows of at least 18 long straight setae, which are arranged pair wise. Propodeum without any hairs; petiole with few suberect setae on dorsal surface of node and abdominal segment III–VIII with irregularly spaced long erect setae. Dense appressed pubescence absent from entire body. Only few body parts bear distinct sculpturing: neck, mesonotum, propodeum, and ventral surface of petiole punctate; lateral surface of propodeum faintly striate.

Holotype and Locality. Brazil: Amazonas, Manaus. Headquarters of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)-Amazônia Ocidental, located at kilometer 28 of highway AM 010; 2°53′S, 59°59′W; elev. 40–50 m; 09 May 2003; col. C. Rabeling; ex leaf litter at dusk, primary tropical lowland rainforest. The holotype is deposited in Museu de Zoologia da Universidade de São Paulo, Brazil (MZSP).

Etymology. The genus name refers to the unknown combination of aberrant morphological characters, which led Stefan P. Cover and Edward O. Wilson to the conclusion that this ant has to be from the planet Mars; hence, the genus name *Martialis* (gr.: "of Mars" or "belonging to Mars"). The species epithet *heureka* (gr.: I found it!) epitomizes the troubled story of the species' rediscovery. Five years after two workers were discovered by M. Verhaagh in a soil sample and subsequently lost, a single worker,

the present holotype, was recollected in a nearby patch of primary rainforest.

Discussion. We assume that the present specimen of *M. heureka* is a worker, because it lacks ocelli, the enlarged mesosoma and the extra sclerites associated with wings (Figs. 1 and 2). In addition, the specimen was collected in the leaf litter suggesting foraging activity usually performed by the worker caste. However, queens with worker-like morphology have been reported from several poneroids and socially parasitic Formicinae and Myrmicinae (26–29). Because we did not perform a dissection of the single specimen no statement can be made about palpal segmentation or internal anatomy.

Inferred Biology. On the basis of the specimen's external morphology, we are able to infer some aspects of the species' biology. The pale integument and the absence of eyes suggest that *M. heureka* lives hypogaeically or in covered low-light environments, like leaf litter or rotting wood. The fact that the first two *M. heureka* individuals were collected in soil core samples during the day, and the present specimen in leaf litter at dusk, supports this hypothesis. Possibly, *M. heureka* surfaces during the night to forage. The unusually enlarged procoxae and long front legs could potentially be an adaptation to prey capture. Presumably, they are used less for digging activities, because the legs are relatively thin and lack the characteristic erect setae of actively digging species. We speculate that *M. heureka* might take advantage of preexisting underground cavities, like hollow rotten roots (Fig. 2). The forceps-like mandible type is not seen in any other ant species. These long, filigree instruments could be used to drag soft prey items out of cavities. Annelids, termites, insect larvae, and other soft-bodied arthropods are possible prey. We do not expect *M. heureka* to prey on heavily sclerotized invertebrates.

Phylogenetic Study. To infer the phylogenetic position of *M. heureka* and to evaluate competing phylogenetic hypotheses, we sequenced 4.2 kb of *Martialis heureka*'s nuclear ribosomal and single-copy nuclear DNA. DNA sequence data were added to a previously published molecular data set of 151 ant species from 20 subfamilies and 11 aculeate outgroup taxa (20), except for four single-copy nuclear genes, which we could not amplify from the limited amount of DNA extract. The phylogeny was inferred using maximum likelihood (ML, 30) and Bayesian inference (BI, 31). Both approaches place *Martialis* as sister to the remaining extant ants and support the monophyly of all ants, including *Martialis* (Fig. 3). *Martialis* did not associate with any extant subfamily in the molecular phylogeny, supporting the designation of the Martialinae as a distinct subfamily on morphological grounds. The basal position of *Martialis* had a Bayesian posterior probability (BPP) of 0.912, a maximum likelihood bootstrap proportion (ML BP) of 0.76 (Fig. 3), and was strongly supported by Bayes factors (BF, 32–34) over the next most strongly supported rooting (Leptanillinae as basal to all extant ants; $2\ln(BF) = -16.4$; Fig. 4). However, the more conservative Shimodaira-Hasegawa (SH, 35, 36) test did not reject the alternative rootings (Fig. 4). Even though the BPP and ML BP were only moderately high, the basal positions of *Martialis* and the Leptanillinae were robust to taxon sampling (Figs. S1 and S2). When *Martialis* was removed from analyses, the leptanillines were recovered as sister to all extant ants. The converse was true when the leptanillines were removed. Because the basal position of neither *Martialis* nor the leptanillines changed in the absence of the other, we suggest that their basal position is not because of a particular taxon-sampling scheme.

The basal position of *Martialis* is not likely caused by long-branch attraction (LBA), as was previously suggested of the leptanillines (20), because *Martialis* has a substantially shorter

Fig. 3. Maximum likelihood (ML) tree with Bayesian posterior probabilities (BPP) and ML bootstrap proportions (ML BP) support values. Tree is rooted on the branch leading to the outgroup, *Pristocera*. The formicoid clade has been collapsed to increase resolution of relationships among basal ant groups (*Martialis*, Leptanillinae, and poneroids). Bipartitions with strong Bayesian support are indicated by blue triangles (BPP = 1.0), green circles (0.95 < BPP < 1.0) or orange rectangles (0.9 < BPP < 0.95).

branch than all outgroup taxa and the Leptanillinae (Fig. 3). Additionally, the ribosomal DNA sequences in this study (87% of our data set) are the most slowly evolving of the genes used by Brady *et al.* (20) (58% of their data set) and are least likely to contribute to LBA artifacts (37). Taxon sampling was also increased by one important lineage over previous studies, breaking the long branch that subtended the extant ants in previous studies (20, 21). However, the reconstruction of the molecular ant phylogeny still faces at least two sources of uncertainty that could introduce analytical artifacts. First, even though *Martialis* breaks the long branch leading to the remaining extant ants, the branch connecting the ingroup to the aculeate outgroup taxa remains long (Fig. 3). Second, the basal ant lineages seem to have originated in a relatively short period (20, 21), potentially making the unambiguous resolution of their relationships quite difficult and sensitive to methodological error. Increased gene and taxon sampling for both ants and outgroup aculeates should reduce the potential for statistical artifacts in future analyses.

Our phylogeny supports paraphyly of the poneroids (Fig. 3). The Ponerinae were strongly supported as sister to the formicoids by Bayesian analysis (BPP = 1), although less strongly by ML bootstrap analysis (ML BP = 68). Relationships among the remaining poneroid subfamilies were more ambiguous. The best estimate from both ML and Bayesian analyses (Fig. 3) has an Amblyoponinae/Agroecomyrmecinae clade basal to all other poneroids, followed by a Paraponerinae/Proceratiinae clade, although support is weak (BPP = 58, ML BP <50). Paraphyly of

Rooting	2 ln(BF)	SH *P*-value
Outgroups / Martialis / Remaining Ants — 1 (ML/MPP)	—	———
Outgroups / Leptanillinae / Martialis/Remaining Ants — 2	-16.4	0.803
Outgroups / Leptanillinae/Martialis / Remaining Ants — 3	-46.9	0.782
Outgroups / Leptanillinae/Martialis, Amblyoponinae, Agroecomyrmecinae / Remaining Ants — 4	-42.7	0.209
Outgroups / Amblyoponinae / Martialis/Remaining Ants — 5	-75.4	0.137
Outgroups / Amblyopone, Adetomyrma, Mystrium / Martialis/Remaining Ants — 6	-60.5	0.131
Outgroups / Leptanillinae/Poneroids / Martialis/Remaining Ants — 7	-67.6	0.178

Fig. 4. Alternative rootings evaluated with Bayes factors (BF) and the Shimo-daira–Hasegawa (SH) test. Constraint trees used to evaluate alternative rootings are depicted on the left. Rootings 1–3 correspond to the first rooting considered by Brady *et al.* (20), but with alternative arrangements of *Martialis* and Leptan-illinae. Rootings 4–7 correspond to Brady *et al.*'s rootings 2–5. For each alternative rooting, the corresponding value of 2ln(BF) is given for the comparison of that rooting against the maximum posterior probability (MPP) rooting 1. Values lower than −10 can be interpreted as strong evidence against the alternative. *P* values resulting from the comparison of alternative rootings to the maximum likelihood (ML) rooting 1 using the SH test are also given. Rootings significantly worse than the ML rooting would have *P* values < 0.05.

the poneroids was recovered, even if the outgroups, *Martialis*, the leptanillines, both the outgroups and *Martialis*, or both the outgroups and the leptanillines were removed from the analysis (Figs. S1 and S2).

Our phylogenetic analyses, combined with the inferred biology of *M. heureka*, suggest that the most basal extant ant lineages are cryptic, hypogaeic foragers, rather than wasp-like, epigaeic foragers (Fig. 3). This finding is congruent with recent molecular studies (20–23), which previously suggested the Leptanillinae, another subfamily of subterranean predators, to be sister lineage to all extant ants. This result has puzzled ant systematists for two reasons. First, Wilson *et al.*'s (16, 17) classic study of the Mesozoic amber ant *Sphecomyrma* postulated that the ancestral ant was a large-eyed, wasp-like, ground forager, creating a strong expectation that the most basal extant ant lineages would also be epigaeic foragers, presumably similar to *Sphecomyrma*. Second, the Leptanillinae share common morphological (10, 38) and behavioral (39, 40) characteristics with the Amblyoponinae, implying the monophyly of this group (20). In contrast, our results and recent molecular systematic studies (20–23) suggest that blind, subterranean, specialized predators, like *Martialis*, the Leptanillinae, and some poneroids, evolved early during ant diversification. We hypothesize, that once these hypogaeic predators adapted to their specialized subterranean environment, their morphology and biology changed little over evolutionary

time because their hypogaeic habitat has likely been ecologically stable and provided a refuge from competition with other, more recently evolved, ants. It is important to note that no definitive statement about the morphology and life history of the ancestral, Mesozoic ant can be derived from our current knowledge about the surviving basalmost ant lineages, because the relative probabilities of evolutionary transitions between epigaeic and hypogaeic habits are uncertain. On the basis of the combined evidence of morphological, phylogenetic, and Mesozoic fossil data, the possibility remains that the ancestral ant was a large-eyed, wasp-like, epigaeic forager (16–19), rather than a blind, specialized, hypogaeic predator.

The Mesozoic fossil *Sphecomyrma freyi* has been interpreted as a representative of the stem-group ants and ant ground-plan characteristics have been derived from its morphology, suggesting that the most recent common ancestor (MRCA) of ants could be similar to tiphioid wasps (16–18). By considering both the topology of our inferred phylogeny (Fig. 3), and the timing of ant diversification presented in previous studies (20, 21), the Sphecomyrminae could alternatively be placed within the subfamilies grouped in the formicoid clade or as stem-group members of the formicoid subfamilies rather than the Formicidae as a whole (16–19). However, the morphology-inferred sister group relationship of *Sphecomyrma* to all extant ants (14) and the absence of hypogaeic ant fossils in Cretaceous deposits (11, 24, 41–50) do not support this hypothesis.

Conclusion. *Martialis heureka* exhibits a mosaic of plesiotypic ant characters and derived morphological adaptations to its hypogaeic predaceous lifestyle. The discovery of *Martialis* and our phylogenetic analyses support the hypothesis that several of the most basal extant ant lineages are hypogaeic foragers, suggesting that hypogaeic foraging evolved at least thrice independently during the early evolution of ants or that the ancestral ant was a hypogaeic forager. These evolutionary scenarios are congruent with the dynastic succession hypothesis for ant evolution (5, 20, 21), proposing a ground-associated ant ancestor, derived from a wasp-like, aculeate predator that radiated into specialized soil, leaf litter, and arboreal habitats potentially coinciding with the diversification of the angiosperms. *Martialis* and other soil-dwelling basal ants might therefore be relicts of early ant diversification, which survived in an environmentally stable refuge habitat, permitting them to retain plesiotypic characteristics because of reduced selective pressures. The exact nature of the ancestral ant remains uncertain, given that the propensity for repeated evolution of a hypogaeic lifestyle may be higher than for reevolution of an epigaeic lifestyle. Interestingly, *M. heureka* is a unique Neotropical representative of an extant, basal ant lineage. This discovery hints at a wealth of species, possibly of great evolutionary importance, still hidden in the soils of the remaining rainforests. Nocturnal leaf litter sampling and subterranean traps would be promising additions to future biodiversity surveys.

Materials and Methods

Photomicrographs were taken using a JVC KY-F70 digital camera mounted on a Leica Z6 APO dissecting scope. Composite images were assembled from 20 sequential photographs with Syncroscopy AutoMontage (v 5.0) software. DNA was extracted from the right front leg using a QIAGEN DNA Micro Kit. Two nuclear ribosomal genes and one single-copy nuclear gene were amplified using conventional PCR methods and sequenced (20). We obtained a total of 4926 aligned bp: 1904 bp for 18S, 2505 bp for 28S, and 517 bp for elongation factor 1 F2. We failed to sequence additional genes from the limited amount of DNA extract. The obtained DNA sequences were aligned with the data matrix of Brady *et al.* (20).

The phylogenetic position of *Martialis* was estimated using both ML and BI. ML trees were inferred using Garli v0.951 (30) with a single model of evolution. Bayesian phylogenies were inferred using MrBayes v3.2 (31) with a four-partition model (1st and 2nd codon positions of EF1αF2, 3rd codon positions

of EF1αF2, 18S, and 28S). Convergence of Bayesian analyses was assessed with the program MrConverge (34). Choices for sequence evolution models (all GTR + I + Γ) were taken from Brady *et al.* (20) for accuracy of comparison with their results and because our taxon-sampling schemes are nearly identical. Adequacy of the model of sequence evolution was evaluated with Bayesian posterior predictive simulation (51) using PuMA v0.903 (see Fig. S3) (52). Phylogenies were also estimated, using identical methods, when excluding *Martialis*, the Leptanillinae, the outgroups, both *Martialis* and outgroups, or both the Leptanillinae and outgroups. Alternative rootings were evaluated using Bayesian bipartition posterior probabilities, Bayes factors (32–34), and the Shimodaira-Hasegawa test (35, 36). For more detail on phylogenetic analyses and results, see *SI Materials and Methods*.

1. Beck L (1971) Soil zoological classification and characterization of the Amazonian rainforest (Translated from German). *Amazoniana* 3:69–132.
2. Fittkau EJ, Klinge H (1973) On biomass and trophic structure of the Central Amazonian rain forest ecosystem. *Biotropica* 5:2–14.
3. Bolton B, Alpert G, Ward PS, Nasrecki P (2006) *Bolton's Catalogue of Ants of the World* (Harvard Univ Press, Cambridge, MA).
4. Agosti D, Johnson NF (May, 2005) *Antbase.* Available at antbase.org. Accessed May 9, 2008.
5. Wilson EO, Hölldobler B (2005) The rise of the ants: A phylogenetic and ecological explanation. *Proc Natl Acad Sci USA* 102:7411–7414.
6. Baroni Urbani C, Bolton B, Ward PS (1992) The internal phylogeny of ants (Hymenoptera: Formicidae). *Syst Entomol* 17:301–329.
7. Brown WL, Jr (1954) Remarks on the internal phylogeny and subfamily classification of the family Formicidae. *Insect Soc* 1:21–31.
8. Hölldobler B, Wilson EO (1990) *The Ants* (Harvard Univ Press, Cambridge, MA)
9. Ward PS (1990) The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): Generic revision and relationship to other formicids. *Syst Entomol* 15:449–489.
10. Ward PS (1994) *Adetomyrma*, an enigmatic new ant genus from Madagascar (Hymenoptera: Formicidae), and its implications for ant phylogeny. *Syst Entomol* 19:159–175.
11. Grimaldi D, Agosti D, Carpenter JM (1997) New and rediscovered primitive ants (Hymenoptera: Formicidae) in Cretaceous amber from New Jersey, and their phylogenetic relationships. *Am Mus Novit* 3208:1–43.
12. Brandão CRF, Martins-Neto RG, Vulcano MA (1989) The earliest known fossil ant (first southern hemisphere mesozoic record) (Hymenoptera: Formicidae: Myrmeciinae). *Psyche* 96:195–208.
13. Verhaagh M (1996) Reasons why the oldest known ant is not an ant (Hymenoptera) (translated from German). *Beitr. Hymenopt.-Tagung* 1996:11.
14. Ward PS, Brady SG (2003) Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). *Invert Syst* 17:361–386.
15. Clark J (1934) Notes on Australian ants, with description of new species and a new genus. *Mem Natl Mus Victoria, Melbourne* 8:5–20.
16. Wilson EO, Carpenter FM, Brown WL, Jr (1967) The first Mesozoic ants. *Science* 157:1038–1040.
17. Wilson EO, Carpenter FM, Brown WL, Jr (1967) The first Mesozoic ants, with the description of a new subfamily. *Psyche* 74:1–19.
18. Wilson EO (1971) *The Insect Societies* (Harvard Univ Press, Cambridge, MA).
19. Taylor RW (1978) Nothomyrmecia macrops: A living-fossil ant rediscovered. *Science* 201:979–985.
20. Brady SG, Fisher BL, Schultz TR, Ward PS (2006) Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proc Natl Acad Sci USA* 103:18172–18177.
21. Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE (2006) Phylogeny of the ants: Diversification in the age of angiosperms. *Science* 312:101–104.
22. Saux C, Fisher BL, Spicer GS (2004) Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae). *Mol Phylogenet Evol* 33:457–468.
23. Ouellette GD, Fisher BL, Girman DJ (2006) Molecular systematics of basal subfamilies of ants using 28S rRNA (Hymenoptera: Formicidae). *Mol Phylogenet Evol* 40:359–369.
24. Bolton B (2003) Synopsis and classification of Formicidae. *Mem Am Entomol Inst* 71:1–370.
25. Latreille PA (1809) *Genera crustaceorum et insectorum secundum ordinem naturalem in familias disposita, iconibus exemplisque plurimus explicata.* (A. Koenig, Paris) Vol 4.
26. Peeters C, Crewe R (1984) Insemination controls and the reproductive division of labor in ponerine ants. *Naturwissenschaften* 71:529–535.
27. Peeters C, Crewe R (1985) Worker reproduction in the ponerine ant. *Ophthalmopone berthouldi*—an alternative form of eusocial organization. *Behav Ecol Sociobiol* 18:29–37.
28. Itow T, *et al.* (1984) The reproductive cycle of the queenless ant *Pristomyrmex pungens*. *Insect Soc* 31:87–102.
29. Brandão CRF (1987) Queenlessness in *Megalomyrmex* (Formicidae: Myrmicinae), with a discussion on the effects of the loss of true queens in ants. *Proceedings of the Tenth International Congress of the International Union for the Study of Social Insects* (Munich, Germany), pp 11–12.
30. Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD dissertation (Univer of Texas, Austin).
31. Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
32. Kass RE, Raftery AE (1995) Bayes factors. *J Am Stat Assoc* 90:773–795.
33. Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL (2004) Bayesian phylogenetic analysis of combined data. *Syst Biol* 53:47–67.
34. Brown JM, Lemmon AR (2007) The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. *Syst Biol* 56:643–655.
35. Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol Biol Evol* 16:1114–1116.
36. Goldman N, Anderson JP, Rodrigo AG (2000) Likelihood-based tests of topologies in phylogenetics. *Syst Biol* 49:652–670.
37. Bergsten J (2005) A review of long-branch attraction. *Cladistics* 21:163–193.
38. Bolton B (1990) The higher classification of the ant subfamily Leptanillinae (Hymenoptera: Formicidae). *Syst Entomol* 15:267–282.
39. Masuko K (1990) Behavior and ecology of the enigmatic ant *Leptanilla japonica Baroni Urbani* (Hymenoptera: Formicidae: Leptanillinae). *Insect Soc* 37:31–57.
40. Gotwald WH, Lévieux J (1972) Taxonomy and biology of a new West African ant belonging to the genus *Amblyopone* (Hymenoptera: Formicidae). *Ann Entomol Soc Am* 65:383–396.
41. Grimaldi D, Agosti D (2000) A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants. *Proc Natl Acad Sci USA* 97:13678–13683.
42. Grimaldi DA, Engel MS, Nascimbene PC (2002) Fossiliferous Cretaceous amber from Myanmar (Burma): Its rediscovery, biotic diversity, and paleontological significance. *Am Mus Novit* 3361:1–71.
43. Engel MS, Grimaldi DA (2005) Primitive new ants in Cretaceous amber from Myanmar, New Jersey, and Canada (Hymenoptera: Formicidae). *Am Mus Novit* 3485:1–23.
44. Perrichot V, Lacau S, Néraudeau D, Nel A (2007) Fossil evidence for the early ant evolution. *Naturwissenschaften*, 10.1007/s00114–007-0301–8.
45. Nel A, Perrault G, Perrichot V, Néraudeau D (2004) The oldest ant in the Lower Cretaceous amber of Charente-Maritime (SW France) (Insecta: Hymenoptera: Formicidae). *Geolog Acta* 2:23–29.
46. Dlussky GM (1987) New Formicoidea (Hymenoptera) of the Upper Cretaceous. *Paleontol Zh* 1987(1):131–135 (Translated from Russian; English translation in *Palaeontol J* 21:146–150).
47. Dlussky GM (1996) Ants (Hymenoptera, Formicidae) from Burmese amber. *Paleontol. Zh.* 1996(3):83–89 (Translated from Russian; English translation in *Paleontol J.* 30:449–454).
48. Dlussky GM, Brothers DJ, Rasnitsyn AP (2004) The first Late Cretaceous ants (Hymenoptera: Formicidae) from southern Africa, with comments on the origin of the Myrmicinae. *Insect Syst Evol* 35:1–13.
49. Wilson EO (1985) Ants from the Cretaceous and Eocene amber of North America. *Psyche* 92:205–216.
50. Ward PS (2007) Phylogeny, classification and species-level taxonomy of ants (Hymenoptera: Formicidae). *Zootaxa* 1668:549–563.
51. Bollback JP (2002) Bayesian model adequacy and choice in phylogenetics. *Mol Biol Evol* 19:1171–1180.
52. Brown JM, ElDabaje R (2008) PuMA: Bayesian analysis of partitioned (and unpartitioned) model adequacy. Available at http://code.google.com/p/phylo-puma. Accessed March 1, 2008.

EVOLUTION