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An Eocene army ant

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Among social insects, army ants are exceptional in their voracious coordinated predation, nomadic life history and highly specialized wingless queens: the synthesis of these remarkable traits is referred to as the army ant syndrome. Despite molecular evidence that the army ant syndrome evolved twice during the mid-Cenozoic, once in the Neotropics and once in the Afrotropics, fossil army ants are markedly scarce, comprising a single known species from the Caribbean 16 Ma. Here we report the oldest army ant fossil and the first from the Eastern Hemisphere (EH), *Dissimulodorylus perseus*, preserved in Baltic amber dated to the Eocene. Using a combined morphological and molecular ultra conserved elements dataset spanning doryline lineages, we find that *D. perseus* is nested among extant EH army ants with affinities to *Dorylus*. Army ants are characterized by limited extant diversification throughout most of the Cenozoic; the discovery of *D. perseus* suggests an unexpected diversity of now-extinct army ant lineages in the Cenozoic, some of which were present in Continental Europe.

1. Introduction

Army ants are preeminent predators of the tropics. Predation among these taxa is famously coordinated; in some species, workers cooperatively forage in massive raiding swarms comprising millions of individuals [1]. In contrast with most ant lineages, army ant colonies are nomadic, and nests are temporarily occupied between phases of travel [2]. Obligate coordinated raiding and colony relocation is central to what has been dubbed the 'army ant syndrome', a suite of traits that typify army ant behaviour and ecology. The syndrome also includes highly specialized wingless queens that participate in colony reproduction through fission [3]. Although historically contentious, recent molecular evidence has confirmed that the army ant syndrome has evolved twice in the Cenozoic: once in the tropical Western (WH) and Eastern (EH) Hemispheres, respectively [4]. Both groups display the behavioural hallmarks of the syndrome, but also possess heavily sclerotized cuticle, robust bauplans, and reduced or absent eyes [5,6]. While WH and EH army ant lineages share all components of the army ant syndrome, there are morphological differences between them: for example, WH genera typically retain reduced eyes in the form of one to a few ommatidia, while EH taxa are generally entirely eyeless [6].

Molecular data indicate that the subfamily Dorylinae—army ants and their allies—originated in the late Cretaceous, while nearly all extant genera originated in the Oligocene and Miocene [4]. WH and EH army ants originated in the Palaeocene between 60 and 57 Ma, and yet radiated into just three to four extant major lineages until the Oligocene–Miocene between 28 and 16 Ma, when they began diversifying into the genera that we see today. Thus, both army ant clades exhibit long initial branches, apparent hallmarks of limited early diversification or significant extinction across the Eocene. Molecular evidence also suggests comparatively recent accelerated rates of evolution in the

army ant clades, which may indicate that convergent evolution of the army ant syndrome took some time to establish in the WH and EH [4].

To date, the only 'true army ant' fossil known is *Neivamyrmex ectopus* from Miocene-age Dominican amber [7]. *Neivamyrmex* today comprises over 100 species and is broadly distributed over the Neotropics, making it a frequently encountered WH genus [6]. Portions of raids occur aboveground in some *Neivamyrmex* species, which may explain its fossilization. There are no army ant fossils described from the EH, and none from the long period of limited diversification or heightened extinction suggested by phylogenetic reconstruction. Here we describe a new taxon from Baltic amber, morphologically distinct from all known doryline fossils, suggesting a more complex phylogenetic and biogeographic history than extant army ants alone divulge.

2. Methods

The type specimen is preserved within Baltic amber (PALE-8463) deposited at the Museum of Comparative Zoology at Harvard University. The specimen was imaged (photography and micro-CT scanning) at the New Jersey Institute of Technology, generating extended focus image composites and a segmented three-dimensional model. We assessed the phylogenetic position of the fossil through both morphological and molecular data. The fossil was appended to a morphological matrix comprising 62 characters and all extant doryline genera and optimized under a parsimony framework; molecular data included two ultra-conserved elements datasets from Borowiec [4] and were optimized simultaneously with the morphological data under a Bayesian framework. To assess the fossil's morphological placement, we performed a principal coordinate analysis using the phylogenetic morphological matrix. A detailed methodology is in the electronic supplementary material; all phylogenetic and morphological data used and generated are provided as electronic supplementary material, data.

3. Results

(a) Systematic palaeontology

Family Formicidae Latreille, 1809

    Subfamily Dorylinae Leach, 1815

    Genus *Dissimulodorylus* Sosiak, Borowiec, & Barden gen. nov.

Diagnosis: Worker. *Dissimulodorylus* can be distinguished from most doryline genera by the combination of lack of eyes, complete pronotomesopleural suture, propodeal spiracle positioned high on the propodeum and single waist segment. These characteristics make it most like *Dorylus*, which can be differentiated by the presence of an impressed pygidial field and lack of fused, dorsoventrally flat, overhanging and triangularly shaped frontal lobes characteristic for this fossil.

Type species: *Dissimulodorylus perseus* sp. nov.

Etymology: From Latin 'dissimulo' meaning to conceal, hide or disregard, in reference to the type specimen eluding description for likely 80 years.

Dissimulodorylus perseus Sosiak, Borowiec, & Barden sp. nov.

Diagnosis: as for genus.

Description: Head: Antennae 11-segmented. Scape 0.48 mm; pedicel roughly twice the length of following segment. Apical antennal segment (as far as it is visible) looks broadly rounded, moderately enlarged relative to others but not a full club. Length of funiculus 1.01 mm. Head capsule rectangular, longer than wide (length of capsule 0.83 mm); breadth of capsule broadly tapers towards oral opening. Ventrolateral margins of head without ridge or lamella. Eyes absent; ocelli absent. Lacking antennal scrobes or frontal carinae; antennal insertions set closely together. Frontal lobes fused and projecting into a triangular structure overhanging the clypeus; clypeus not visible. Lateroclypeal teeth and parafrontal ridges absent or not discernible. Mandibles falcate: elongate and narrow, lacking teeth and tapering midway suddenly towards sharp apex. Cuticle shining with sparsely punctate sculpturing; scattered short and broad setae over dorsal surface; some setae appear apically spatulate. Cuticular colour medium brown, though may be affected by preservation.

Mesosoma: Weber's length 1.21 mm. Pronotal flange not separated from collar by distinct ridge. Pronotomesopleural suture complete, continuous with promesonotal suture. Enlarged propleuron in lateral view. Mesonotum is reduced to small flanged sclerite angled upwards anteriorly; metanotal groove absent. Mesopleuron elongate, lacking transverse groove or impression. Mesosoma dorsolaterally immarginate. Propodeal cuticle imbricate; fine-short dark setae scattered dorsally but not as thickly as on dorsal pronotal surface. Propodeal spiracle large and circular, positioned high and anteriorly on sclerite. Propodeal declivity rounded; posterior surface shorter than dorsal surface; propodeal lobe present and upturned slightly. Metapleural gland bulla visible through cuticle. Propleural cuticle appearing imbricate, though may be a function of desiccation during preservation. Dorsally pronotum covered in short-fine dark setae; pronotal cuticle also imbricate; promesonotal suture well-defined and unfused. Cuticular colour as in head. Coxae large and boxy; procoxa 0.39 mm; mesocoxa 0.33 mm; metacoxa 0.34 mm; coxa cuticular texture imbricate and covered with fine pale setae. Profemur cuticle smooth and shining; profemur length 0.76 mm; single pectinate protibial spur; protarsus mostly missing. Mesofemur cuticle smooth and shining, scattered fine light setae; mesofemur length 0.84 mm; distal half of mesotibia missing. Metafemur cuticle smooth and shining; metafemur length 1.06 mm; metatibia bearing single large simple spur; metatarsus long, first segment roughly as long as next four segments combined, with segments steadily shrinking in length distally; metatarsus covered in fine-long setae; metatarsal claws large, simple and sharply curved.

Metasoma: Petiole immarginate, dorsally broadly triangular tapering anteriorly, laterally broadly rounded with sloping anterior face and overhanging posterior face. Attachment to propodeum elongate and constricted; ventral surface with spine positioned anteriorly and pointing anteriorly. Helcium positioned ventrally. Abdominal segment III smaller relative to segment IV, girdling constriction present between segments, though constriction not especially deep. Pygidium and hypopygium not clearly visible in specimen or scan; no cuticular or conspicuous peg-like teeth on the pygidium. Sting present, mostly retracted. Cuticle mostly smooth and shining with sparsely punctate sculpturing. Cuticular colour as in head and mesosoma, slightly darker.

Type material: Holotype PALE-8463 deposited within the Museum of Comparative Zoology (MCZ) at Harvard University.
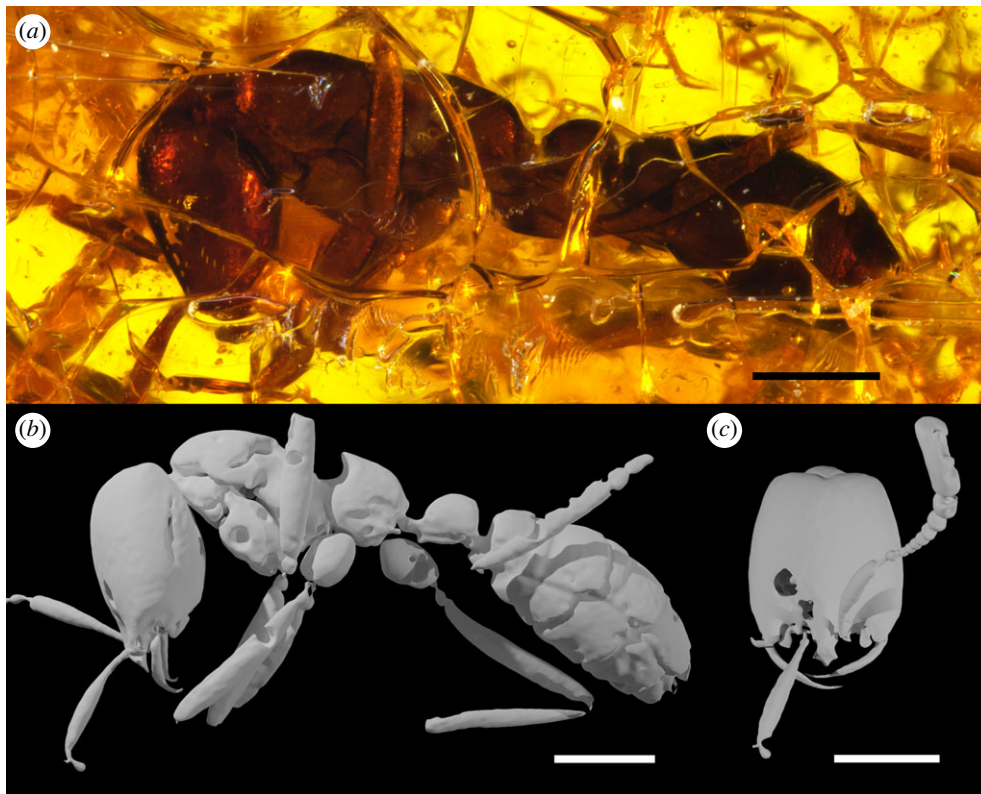
Figure 1. Specimen PALE-8463 from Eocene age Baltic amber. (*a*) Photomicrograph of lateral profile view of *D. perseus*. (Bottom) Micro-CT scan reconstruction of *D. perseus*, in (*b*) lateral and (*c*) frontal view. Scanning primarily resolved internal 'void space' air trapped within the insect cuticle, apparent holes in specimen reflect imperfect infilling of gas. Scale bars 0.5 mm. Specimen images are © President and Fellows of Harvard College, Museum of Comparative Zoology, Harvard University.

Etymology: In reference to the mythological Greek hero Perseus, a warrior who defeated Medusa without the use of direct sight (figure 1).

(b) Phylogenetic reconstruction and morphospace analysis

We recover *D. perseus* as nested within 'true' EH army ants across optimality criteria and datasets (figure 2*a*). Our morphological equal and implied weights parsimony reconstruction recovers EH and WH as monophyletic, reflecting the strong phenotypic convergence of these lineages (electronic supplementary material, figures S1 and S2). Army ant monophyly is also recovered in our 109 loci (44 kb) UCE + morphology Bayesian Inference search, a result that is notably sensitive to search parameters and input data [4] (electronic supplementary material, figure S3). Our 'slow-evolving' 100 supergene loci (178 kb) + morphology Bayesian Inference topology is in-line with previous hypotheses generated from this dataset [4]; *D. perseus* is recovered among a polytomy with *Aenictogiton* and *Dorylus* with modest support, but monophyletic with all EH army ants with strong support (figure 2, electronic supplementary material, figure S4). Distance-based morphospace analysis highlights the affinities among *Dorylus*, *Aenictogiton* and *D. perseus*, to the exclusion of most other doryline taxa and WH army ants (figure 2*b*).

4. Discussion

We find *Dissimulodorylus perseus* is nested within the EH army ant clade as monophyletic with *Aenictogiton* and

Dorylus, sharing the most recent common ancestor of all extant EH army ant taxa. This phylogenetic relatedness coupled with molecular evidence [4] suggests that the army ant syndrome was present in the most recent common ancestor of the clade; it is therefore likely that *D. perseus* shared these characteristics of obligate cooperative foraging, nomadic nesting habits and wingless specialized queen castes. Additionally, *D. perseus* is morphologically akin to modern army ants and shares several traits likely indicative of the syndrome. Similar to modern EH army ants, it lacks eyes, suggesting that it would have closely followed pheromone trails [9]; its mandibles are sharply pointed and lack a masticatory margin, consistent with raid-based predation [10]; and it has a well-developed metapleural gland, suggesting large colonies and partial subterranean living [11].

Crown group army ant genera originated in the late Oligocene and Miocene, well after the fossil was formed. While the common ancestor of the EH army ant clade diverged prior to *Dissimulodorylus*, the fossil predates or coincides with accelerated rates of phenotypic evolution associated with the diversification of modern army ants [4]. Molecular phylogenetic reconstruction has demonstrated that there are approximately 30 Myr between the most recent common ancestor of the EH army ant clade and the origin of *Dorylus* and *Aenictogiton*, during which there is no cladogenesis of extant lineages [4]. *Dissimulodorylus* is unlikely to represent a lineage within crown *Dorylus*, which most recently has been dated to be 11–22 Ma old [4], although an early molecular dating estimate put the age of the genus at 46 Ma [12]. Considering the subfamily's long-branch lengths in the context of *Dissimulodorylus*, it seems plausible that other stem army ant lineages diverged and went extinct during the mid-Cenozoic. This morphologically modern
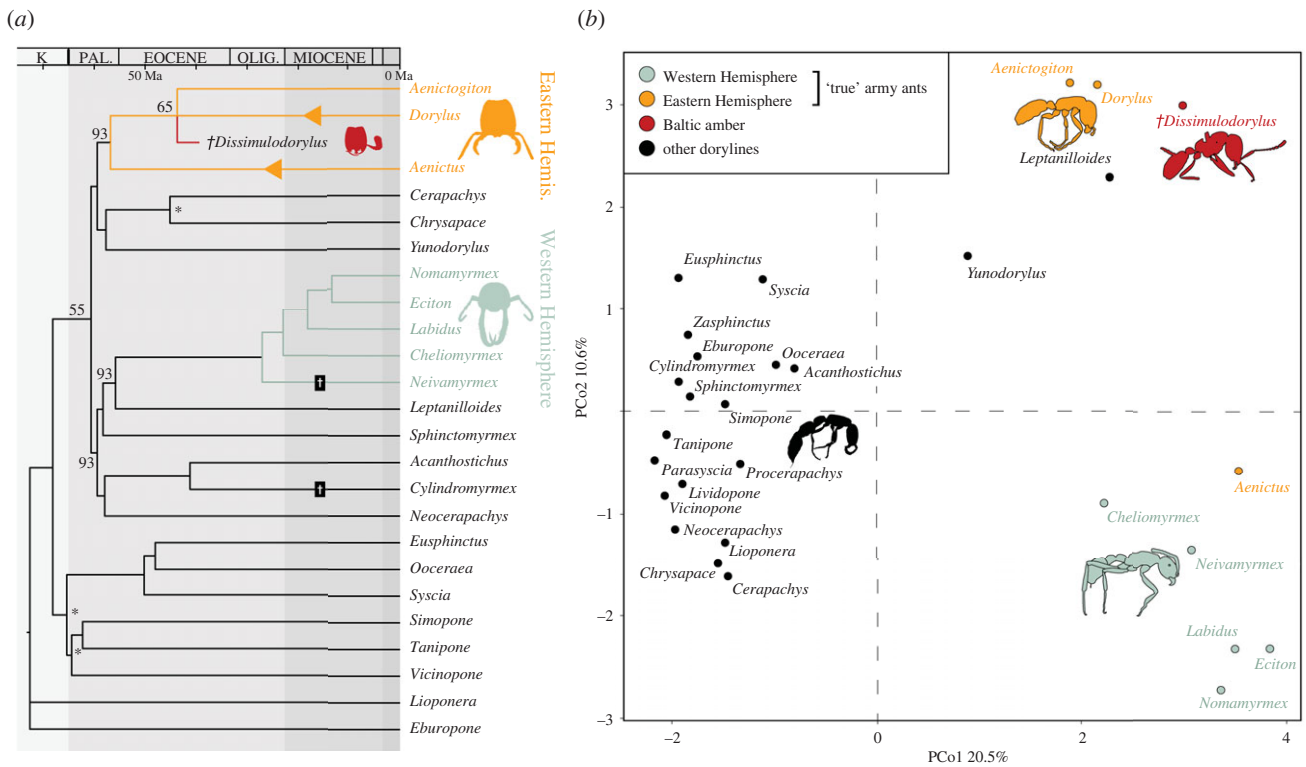
Figure 2. Phylogenetic and phenotypic position of *Dissimulodorylus perseus* relative to extant dorylines. (*a*) Consensus tree resulting from combined analysis of 178 662 (100 supergene loci obtained using weighted statistical binnings [4,8]) molecular and 62 morphological characters under Bayesian Inference. Nodes with posterior probability support < 0.95 noted with numerical values. Node ages reflect mean divergence time estimates from Borowiec [4]; nodes denoted with an asterisk were not recovered in divergence dating estimation of Borowiec [4] and so are arbitrary. Triangles denote estimated crown ages from Borowiec [4]. Fossils belonging to extant genera are indicated with the dagger (†) symbol. (*b*) Principal coordinates morphospace derived from Euclidean distance matrix of binary morphological characters included in phylogenetic reconstruction.

fossil illustrates previously undiscovered army ant diversity in the Cenozoic.

Like *Dissimulodorylus*, there are other ant lineages known from Baltic amber that are today restricted to the Afrotropics and southeastern Asia. Two clear examples are *Gesomyrmex* and *Oecophylla*. *Gesomyrmex hoernesi* is described from Baltic amber [13], and several other species of *Gesomyrmex* are known from various compression fossil deposits in Europe and Russia [14–16], while all extant species are present in Asia; indeed, the fossil diversity of *Gesomyrmex* (11 species) outstrips its present-day diversity (seven species). Similarly, present-day diversity of *Oecophylla* comprises two species, *O. smaragdina* in southeastern Australasia and *O. longinoda* in the Afrotropics, but its historical diversity was much broader, comprising 16 species across Cenozoic Europe [17–21].

There are myriad other examples from the overlap of Eurasian paleoentomofauna and present-day Afrotropical–Australasian faunas: 39 genera present today in these regions can also be found in these fossil assemblages [22]. Not only are generic compositions similar, so too are species richness and composition between the two faunas, potentially suggesting a broad range of early EH army ant diversity in the Eocene [22]. During the Eocene, Europe was overall warmer and wetter than it is today, creating a large span of suitable habitat across Eurasia. Throughout the latter half of the Cenozoic, however, repeated warming and cooling cycles were likely inhospitable to assemblages adapted to tropical climes [23]. *Dissimulodorylus perseus* is another piece of evidence suggesting that present-day Afrotropical and southeast Asian ant assemblages are relict distributions of pan-Eurasian assemblages that diversified during the mid-Cenozoic.

Perhaps due to frequent subterranean specialization, fossil dorylines are lacking. Five species are known from Dominican amber (approx. 16 Ma), and three species known from Baltic amber (approx. 34 Ma). Three Dominican amber species are from the genus *Cylindromyrmex*, whose extant representatives are typically arboreal [24,25]. The other two species are from extant subterranean or partially subterranean genera, *Acanthostichus* and *Neivamyrmex* [7,26]. Baltic amber species are all described from the extinct genus *Procerapachys* [27,28] with uncertain phylogenetic status as many specimens have been lost; some species appear to be similar to the extant arboreal genus *Chrysapace*, while others appear morphologically distinct [6]. Regardless, considering their morphology, these species are likely not army ant dorylines [6,28].

Dissimulodorylus perseus demonstrates that amber, though often considered biased towards arboreal species, can capture a wide range of arthropod ecologies. Evidence from fossil ant assemblages with extant congenerics illustrates that surface- and subterranean-dwelling species are often captured in resin [22]. Extant simulations of resin capture using sticky traps on resin-producing trees has also shown that insects from a broad range of ecologies are captured [29,30]. Army ant lifestyles are typically partially subterranean, with minimal interaction with arboreal habitats, but the fossilization of *D. perseus* instills hope for the preservation of a range of cryptic subterranean species.

an accompanying table listing all supplementary files and brief descriptions of these files. The CT data can be found at the Dryad Digital Repository: https://doi.org/10.5061/dryad.d7wm37q4c [31].

The data are provided in the electronic supplementary material [32].

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