E-ARTICLE

Trait-Based Paleontological Niche Prediction Recovers Extinct Ecological Breadth of the Earliest Specialized Ant Predators

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ABSTRACT: Paleoecological estimation is fundamental to the reconstruction of evolutionary and environmental histories. The ant fossil record preserves a range of species in three-dimensional fidelity and chronicles faunal turnover across the Cretaceous and Cenozoic; taxonomically rich and ecologically diverse, ants are an exemplar system to test new methods of paleoecological estimation in evaluating hypotheses. We apply a broad extant ecomorphological dataset to evaluate random forest machine learning classification in predicting the total ecological breadth of extinct and enigmatic hell ants. In contrast to previous hypotheses of extinction-prone arboreality, we find that hell ants were primarily leaf litter or ground-nesting and foraging predators, and by comparing ecospace occupations of hell ants and their extant analogs, we recover a signature of ecomorphological turnover across temporally and phylogenetically distinct lineages on opposing sides of the Cretaceous-Paleogene boundary. This paleoecological predictive framework is applicable across lineages and may provide new avenues for testing hypotheses over deep time.

Keywords: paleoecology, ants, morphology, machine learning.

Introduction

Estimating the ecological niche occupation of extinct taxa is a central component of paleontology. The putative ecologies of extinct organisms are routinely incorporated into analyses of extinction risk, paleoenvironmental reconstruction, and lineage evolutionary history (Palmqvist et al. 2003; Benson et al. 2014; Frederickson et al. 2018). Even as aspects of extinct species' niche occupation may be reliably inferred by the preservation of individual traits in fossil specimens, organismal ecology remains multifaceted. Morphology may often—though not always (Miller et al. 2017)—reflect ecology across entire phenotypes (Williams 1972; Losos 1992), and phenotypes may be linked to multiple aspects of an organism's ecological niche spanning habitat, diet, and interactions. These ecologically linked body plans—ecomorphs— are found in such disparate taxa as fish, reptiles, arthropods, and mammals (Saunders and Barclay 1992; Barton et al. 2011; Gerry et al. 2011; Saunders et al. 2013; Figueirido et al. 2019). The relationship between ecological niche and multitrait morphology can also be leveraged to estimate paleoecologies. In lineages with surviving relatives, extant taxa may serve as data-rich analogs for ecological niche estimation: however, the partial preservation of many fossil specimens and aberrant phenotypes in some extinct lineages may reduce the utility of extant-to-extinct comparisons.

Across vertebrate species, limb anatomy is a predictor of locomotion, prey items, and substrate behavior. In particular, the forelimb anatomy of carnivores has been used to predict the likely predatory habits and prey size of extinct carnivorous mammals and mammaliaforms (Ercoli et al. 2012; Meloro and Louys 2014; Figueirido et al. 2016; Dunn et al. 2019; Jenkins et al. 2020; Lungmus and Angielczyk 2021). Other examples of the morphology of extant species being used in the prediction of extinct species' ecology include estimating diet in extinct raptor species from present-day birds of prey (Hertel 1995), predicting prey items and modes of scavenging in extinct crocodyliforms from extant crocodyliform snout morphology (Drumheller and Wilberg 2020), approximating arboreal behavior in extinct primates (Rector and Vergamini 2018), and predicting habitat preferences in fossil *Anolis* lizards, using ear canal shape

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(Dickson et al. 2017). In many cases, these methods incorporate isolated body parts rather than full-body morphology, potentially because trait ecomorphology has been more intensely studied in vertebrates than in other animal taxa and is thus more well defined. However, morphometric analyses of full mammalian skeletons have been used to predict the locomotion mode of various Mesozoic mammaliaforms (Chen and Wilson 2015; Meng et al. 2017).

Attempts to predict paleoecology from extant morphology frequently use techniques such as canonical correlations analysis or canonical variate analysis and, in particular, linear discriminant analysis (Hertel 1995; Janis and Figueirido 2014; Meloro and Louys 2014; Dickson et al. 2017; Rector and Vergamini 2018; Dunn et al. 2019). These methods maximize variation in the measured traits between predetermined classes in morphospace. The fossil specimen's most likely ecology is then determined by proximity to each class mean in this constructed morphospace (Strauss 2010). These approaches are powerful tools for establishing sets of traits most strongly associated with predetermined classes but are limited to linear relationships only among measured traits, which may restrict accuracy by failing to incorporate nonlinear predictive relationships between traits.

While most predictive paleoecology studies have focused on vertebrate paleoecology, minimal attention has been paid to these approaches in invertebrates, particularly insects. Many extant insect lineages reach back to the Mesozoic or Paleozoic and are highly ecologically diverse. One example is the ants, which arose between ~150 and 100 Ma (Brady et al. 2006; Moreau et al. 2006; Borowiec et al. 2019). With over 15,000 species comprising a significant component of terrestrial biomass, ants are globally ubiquitous, speciose, and present in most postproducer ecological niches (Hölldobler and Wilson 1990; Bolton 2021). Importantly, despite this ecological diversity, ants are also morphologically conserved with respect to broad body plan functionality and possess a rich fossil record extending from 100 Ma to present day. A majority of ant fossils are known from fossil amber, which often preserves entire specimens with high fidelity. Because of their well-defined homology and uniquely preserved fossil history, extinct ants are strongly suited for testing paleoecological niche prediction methods.

The earliest known ant fossils date to the Early–Late Cretaceous transition and comprise extinct stem lineages that began to diversify prior to the most recent common ancestor of all living ants. While crown lineages diversified concomitant with these stem lineages, all stem lineages became extinct near the end of the Cretaceous, while crown lineages persisted into the Cenozoic, exemplifying a distinct faunal turnover across the Cretaceous-Paleogene boundary. Cretaceous fossils have fueled speculation on the ecological occupation of the earliest ants because these taxa have bearing on the evolution of eusociality more broadly. Early speculation on Cretaceous ant ecology posited that these early species were unlikely to construct nests but instead used already present cavities in soil and wood, on the basis of assumptions that these early species were primitively eusocial and thus unlikely to cooperate as well in nest building or that their mandible morphology was prohibitively specialized to allow for nest construction (Wilson et al. 1967; Wilson 1987a, 1987b; Dlussky 1996; Grimaldi and Agosti 2000; Engel and Grimaldi 2005). On the basis of their presumed wasp ancestors, they were additionally argued to be predators (Wilson 1987a, 1987b; Dlussky 1996). As the taxonomic diversity of extinct ant species increased with the discovery of new fossils, paleoclimate and phylogenetic reconstructions suggested that early ants occupied soil and leaf litter microhabitats in newly emerging angiosperm forests (Wilson and Hölldobler 2005; Moreau et al. 2006; Perrichot et al. 2008; Moreau and Bell 2013). Phylogenetic reconstructions using extant lineages have also recovered ant ancestors as potentially hypogeic soil dwellers (Lucky et al. 2013). While there have been secondary inferences regarding the ecology of the earliest ants, no fossil-derived data have yet been included in the reconstruction of ancient ant ecology.

Haidomyrmecines, or hell ants, are an enigmatic and morphologically aberrant extinct subfamily of ants comprising 16 described species and 10 genera (Perrichot et al. 2020). Hell ants occupy a stem group position relative to modern ants and are frequently recovered as sister to all other extinct and extant ants (Barden and Grimaldi 2016; Barden et al. 2020). They persisted throughout the mid-to-late Cretaceous—as evidenced by amber fossils ranging from 100 to 78 Ma on three different continents in Canada, Myanmar, and France (Dlussky 1996; Perrichot et al. 2008; McKellar et al. 2013)—and are hypothesized to have undergone extinction concomitant with the early radiation and diversification of extant lineages. These ants are morphologically unusual in having vertically articulating mandibles, unlike the horizontal alignment of modern ants. Remarkably, hell ants possess an array of horn-like cranial appendages that have been directly observed to facilitate solitary predation through fossil remains (Barden et al. 2020): haidomyrmecines captured prey individually by articulating their mandibles against their horns. Hell ants have been hypothesized as arboreal predators, considering the potential difficulty of substrate manipulation with their vertically aligned mandibles and frequent preservation in amber, potentially indicating close proximity to tree resin (Dlussky 1996; Barden and Grimaldi 2012; Lattke and Melo 2020). Arboreality may be a risk factor in cataclysmic mass extinction events, given the dependency on habitats that are more exposed to extreme weather fluctuations and other extreme events, such as wildfires (Field et al.

2018). If hell ants were indeed arboreal, it may have been a contributing factor to their extinction toward the end of the Cretaceous.

Were hell ants indeed arboreal, potentially increasing their risk of extinction? How do the ecologies of the earliest ants compare with extant lineages today? Did functional or ecological succession accompany ant faunal turnover from the Cretaceous to the Cenozoic? Here, we use a wide-ranging extant morphometric dataset spanning over 160 species that has previously demonstrated a quantitative link between morphology and ecology to predict the paleoecology of several hell ant species. Using a supervised machine learning classification algorithm—random forest—we predict foraging niche, nesting niche, and functional role for hell ants. With these predictions, we reconstruct known ecomorphological space for haidomyrmecines and compare these ecological occupations with those of extant lineages of specialized solitary predators. Our results demonstrate repeated filling of functional niche space across phylogenetically and temporally distant lineages. This approach provides a generalizable framework for paleoecological estimation beyond either single-trait interpretations or subjective interpretations from taxonomic expertise, opening future directions for the reconstruction of extinct communities and ecosystems.

Methods

Extant Morphological Data Sampling and Ecological Niche Binnings

We sampled extant ant morphological data following the protocol of Sosiak and Barden (2021; fig. 1). Our extant dataset spans 15 subfamilies, 113 genera, and 167 species, sampling three specimens per species where possible and measuring as many conspecifics as were present in museum collections otherwise. Polymorphic species are represented by the media caste—or caste representing a morphological midpoint between small and large workers—and species with specialized castes are represented by nonspecialized minor workers. There is currently no evidence for specialized hell ant worker castes, and specialized worker castes are not a synapomorphy of crown ants, informing our choices of caste sampling.
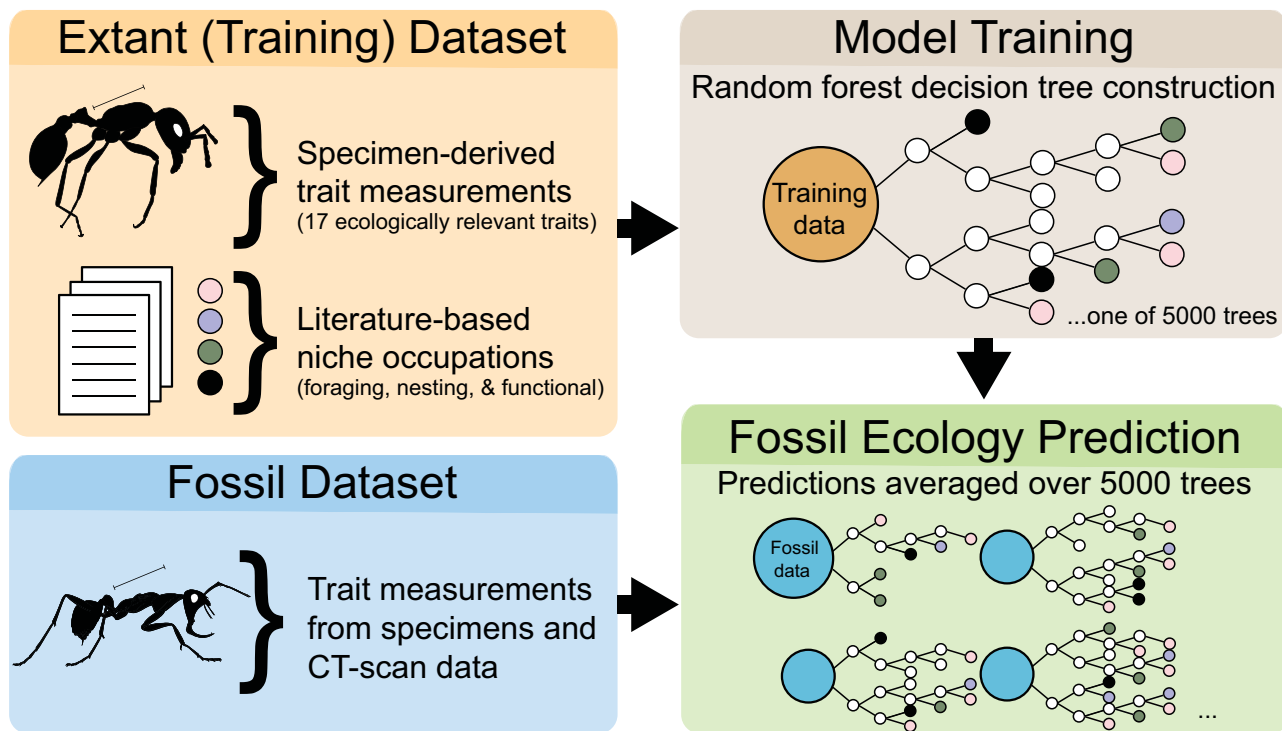

Figure 1: Diagrammatic workflow of predictive model development and testing. A comprehensive morphometric dataset of extant ants was compiled; species were binned according to various ecological niche aspects based on surveys of the literature. Random forest models were then trained on subsets of the original dataset. Homologous traits were measured on fossil ant specimens; when available, traits were measured from CT reconstructions and otherwise were measured under light microscopy. Finally, the pretrained random forest models were used to predict extinct ecology from fossil morphometric datasets.

Our morphometric sampling comprised linear measurements of 12 cephalic traits and five postcephalic traits (table 1). Most sampled traits have been previously linked to ecology (Weiser and Kaspari 2006; Yates and Andrew 2011; Yates et al. 2014; Gibb et al. 2015). All measurements were conducted on point-mounted specimens under stereo microscopy. Because linear measurements alone may not fully capture important trait variation, and body size in diverse species can drive most variation in a dataset and potentially mask other important contributors, we created three datasets: one comprising raw linear measurements, one comprising shape ratios calculated from the linear measurements, and one size-corrected dataset comprising log shape variables and log body length or Weber's length (Mosimann 1970). A list of all shape ratio traits with associated definitions may be found in table S1 (tables S1–S52 are available online).

All specimens were assigned a binning from each of three ecological niche aspect categories, following the protocol of Sosiak and Barden (2021): functional role (referring to the diet of the species), nesting niche (the type of nest and stratum in the environment), and foraging niche (the stratum in the environment where the species forages for food; fig. 1; table 2). Specimen binnings were assigned on the basis of literature surveys. When any particular aspect of a species' ecological niche was uncertain, the species was assigned an unknown binning and excluded from further model training. We found 35 total observed combinations of niche binnings across all niche aspects and specimens; we additionally collapsed these 35 combinations into 10 simplified ecomorph syndromes (based on ecological and morphological overlap) to evaluate whole-body ecomorphological correlates. A list of all ecological niche aspect combinations and ecomorph

Table 1: All morphological traits measured, with description of measurements taken and known ecological significance of traits

Trait	Description of measurement	Known ecological significance
Head width (HW)	Taken in frontal view along widest axis of head capsule, excluding eyes	Mandibular musculature of workers (Kaspari 1993); size of spaces workers can move through (Sarty et al. 2006)
Head length (HL)	Medially from anterior margin of clypeus to vertex of head capsule in frontal view	Size of spaces workers can move through (Kaspari and Weiser 1999)
Eye length (EL)	Measured along longest axis of eye	Foraging behavior and foraging period (Weiser and Kaspari 2006)
Mandible length (lateral profile view; MLP)	From point of insertion to apical-most tooth of mandible	Diet (Fowler et al. 1991)
Anteroposterior eye position (three measurements taken: LHL, ELA, ELP)	Taken in lateral view: length from midpoint of eye to anterior clypeal margin (ELA); length of head from midpoint of eye to posterior margin (ELP); total lateral head length (LHL) used to calculate eye position ratios	Foraging and diet (Fowler et al. 1991); habitat stratum (Gibb and Parr 2013)
Dorsoventral eye position (three measurements taken: HH, EHD, EHV)	Taken in lateral view: height of head from midpoint of eye to dorsal margin of head (EHD); height of head from midpoint of eye to ventral margin of head (EHV); total head height (HH) used to calculate eye position ratios	Related to foraging and diet (Fowler et al. 1991); habitat stratum occupied (Gibb and Parr 2013)
Mandible length (frontal view; MLF)	From point of clypeal insertion to apical-most tooth of mandible	Diet (Fowler et al. 1991)
Scape length (SL)	From antennal socket to distal margin of scape	Chemosensory; detection of pheromone trails (Weiser and Kaspari 2006)
Weber's length (WL)	Taken in lateral view from anterodorsal margin of pronotum to posteroventral margin of mesosoma	Established proxy for worker body size (Weber 1938)
Procoxal length (PL)	From articulation point with propleuron to distal tip of procoxa	Not applicable
Mesosoma height (MH)	Taken at right angle to Weber's length from ventral margin of propleuron to dorsal margin of pronotum	Not applicable
Pronotal width (PW)	Measured at widest point of pronotum when viewed dorsally	Body mass of workers (Kaspari and Weiser 1999)
Metafemur length (ML)	Measured from articulation point with trochanter to distal tip of metafemur	Foraging speed and habitat complexity (Feener et al. 1988)

Table 2: Ecological niche aspect binning abbreviations, definitions, and exemplar taxa

Binning designator	Definition	Exemplar taxa
Functional role:		
GP	Generalist predator: broad taxonomic diet	*Odontomachus, Diacamma, Harpegnathos*
SP	Specialist predator: obligate feeding on specific taxon (e.g., termites)	*Acanthostichus, Megaponera, Simopelta*
Om	Omnivorous: prey items, plant matter, etc.	*Paraponera, Camponotus, Iridomyrmex*
Py	Phytophagous: extrafloral nectaries, herbivory, etc.	*Pseudomyrmex, Tetraponera, Myrmelachista*
Fg	Fungus growing	*Cyphomyrmex, Trachymyrmex, Atta*
Tr	Trophobiotic: symbiotic relationship with other insects (homopteran secretions, etc.)	*Acropyga, Melissotarsus, Rhopalomastix*
Gn	Granivorous: seed harvesting	*Acanthomyrmex, Pogonomyrmex, Veromessor*
Mh	Mushroom foraging	*Euprenolepis*
Nesting niche:		
Cn	Carton nesting: structured nests from plant material in trees and shrubs	*Oecophylla, Azteca, Liometopum*
Gr	Ground nesting: nests in dirt mounds, under stones, rock cracks, etc.	*Platythyrea, Formica, Pheidole*
Lg	Lignicolous: nests in twig and tree cavities	*Pseudomyrmex, Simopone, Cylindromyrmex*
Ll	Leaf litter nesting: nests in leaf litter interstitial space, rotten wood, etc.	*Strumigenys, Discothyrea, Typhlomyrmex*
Sb	Subterranean nesting	*Leptanilloides, Leptanilla*
Foraging niche:		
Ab	Arboreal: in and on trees and shrubs	*Daceton, Tetraponera, Crematogaster*
CR	Column raiding: cooperative, nomadic, or raiding predation	*Simopelta, Dorylus, Eciton*
Eg	Epigeic: active foraging on ground surface	*Leptomyrmex, Rhytidoponera, Myrmecocystus*
Ll	Leaf litter: within interstitial spaces in leaf litter	*Discothyrea, Amblyopone, Heteroponera*
Sb	Subterranean: underground	*Acropyga*

syndromes with associated definitions may be found in table S2.

Fossil Morphological Data Sampling

We measured fossil hell ant specimens using a combination of stereo microscopy and reconstructions of X-ray microcomputed tomography (micro-CT) scans (fig. 1). All hell ant specimens were from Kachin amber (99 Ma). Twenty specimens from 16 species and morphospecies were measured under stereo microscopy. We submerged the amber specimens in water to reduce light distortion; some measurements were not possible because of specimen positioning. Three specimens were micro-CT scanned and reconstructed for subsequent measurements: two species of hell ant (*Haidomyrmex scimitarus* and *Linguamyrmex vladi*) and a *Pseudomyrmex macrops* specimen from Dominican amber (16 Ma) to assess the reliability of CT scan–based data. Congeners of the Dominican *Pseudomyrmex* fossil are extant today and their ecology is consistent across the genus and well characterized. The *H. scimitarus* (specimen AMNH Bu-FB80) and *L. vladi* (specimen AMNH BuPH-1) specimens were scanned at the American Museum of Natural History Microscopy and Imaging Facility, using a GE phoenix v|tome|x s240 60-kV CT scanner. Specimen AMNH Bu-FB80 was imaged at 180 μA for 5-s exposures and a voxel size of ∼8 μm, and specimen AMNH BuPH-1 was imaged at 250 μA for 1-s exposures and a voxel size of ∼3 μm. The *P. macrops* specimen (AMNH DR-14-1021) was imaged at the New Jersey Institute of Technology York Center, using a Bruker SkyScan 1275 at 60 kV and 150 μA for 1-s exposures with a subsequent voxel size of ∼3.5 μm. Volume reconstruction of the X-ray images was conducted in 3D Slicer version 4.11 (Fedorov et al. 2012) using the segmentation modules; still images of the reconstructed specimens were subsequently imported into ImageJ (Abrà-moff et al. 2004) for linear measurements to retain consistency with measurements taken under stereo microscopy. Postcranial morphology of hell ants is similar to extant ant species, while cranial morphology is highly aberrant. To ensure that we were assessing morphological variation in terms of both homologous and functional morphology, we partitioned data according to homologous or functional measurements; a full discussion of homologous versus functional morphology may be found in the supplemental PDF.

Because of limitations measuring specimens directly from amber fossils, we produced two fossil morphometric datasets: one incomplete dataset that excluded a subset

of traits for all specimens and one with all measurements included. The incomplete dataset lacked the frontal head length, head width, frontal mandible length, and pronotal width measurements. These measurements are often difficult to accurately capture because amber fossils are typically prepared to expose a clear lateral profile of any specimen, leaving the dorsal and frontal margins of the amber rounded and distorted. Twenty hell ant specimens were included in this incomplete dataset. The complete dataset comprised the proof-of-concept fossil *P. macrops* specimen and three hell ant specimens: *Dhagnathos autokrator*, *H. scimitarus*, and *L. vladi*. While the majority of specimens included were workers, two of the specimens—the complete *D. autokrator* and *H. scimitarus*—were represented by alate (winged) and dealate (wings shed) queens, respectively. We included queens for two reasons: (1) hell ant queens are hypothesized to have actively foraged and hunted in early colony foundation and so likely occupied a similar ecological niche to the workers of the species; and (2) fossilized worker specimens are not known for many hell ant species and are entirely unknown for the genus *Dhagnathos*. While we have no comparison for *Dhagnathos* workers, we include a *H. scimitarus* worker in the incomplete morphometric dataset, allowing us to compare the accuracy of the model in predicting queen and worker ecology. A full list of all specimens included with information pertaining to their sampling methods and castes can be found in table S3; all morphometric data for fossil specimens are available in Zenodo (https://doi.org/10.5281/zenodo.7897553; Sosiak et al. 2023).

To ensure that morphological diversity for traits measured from fossil species are within the bounds of extant morphological diversity, we conducted principal component analyses (PCAs) to compare morphospace occupation of hell ants relative to extant lineages. We conducted separate PCAs for all three measurement datasets. All PCAs were implemented in R packages corrplot (Wei et al. 2017) and FactoMineR (Lê et al. 2008).

Model Training and Testing

We implemented random forest analysis, a supervised machine learning algorithm, to delimit species into ecological niche binnings by morphology. Random forest algorithms rely on an ensemble of decision trees, with each tree providing a vote for a majority consensus determining the predicted class (Breiman 2001). Each decision tree is built through iteratively sampling the training dataset with replacement, with each internal node or split in the tree being selected from the variable contributing highest accuracy from a randomly subsampled set of predictor variables. As the trees are iteratively built, each tree provides a vote on the most likely class for each sample in the training dataset;

the algorithm then uses the vote consensus to predict class for each sample. The iterative sampling or bagging of the training dataset to build the trees and the randomly selected subset of variables at each node ensure that the decision trees are uncorrelated and that the model is not overfitted to the training dataset. Throughout model construction for each tree, random forest algorithms iteratively sample two-thirds of the provided training dataset for training the model and one-third of the provided dataset for testing the model; error rate across these iterations is then averaged to provide out-of-bag error rate for the model. This removal of testing data during bootstrapping eliminates the need for a priori separation of a testing and training dataset, incorporating all collected data. Model parameters include *ntree* (the number of trees in the ensemble model) and *mtry* (the number of variables randomly selected to be tested at each split).

While other supervised machine learning or dimension reduction techniques are more commonly used in morphology-based paleoecological prediction, random forest has recently been shown to outperform linear predictive approaches with respect to morphology (Pigot et al. 2020; Sosiak and Barden 2021), hence our use here. We trained our random forest models using our extant ant morphometric dataset with known ecological niche binnings (fig. 1). We constructed random forest models for the linear measurements, shape ratios, and log shape variable datasets separately for each ecological niche aspect and overall ecomorph syndrome, allowing for granular classification of ecological niche and more synthesized classification. Model parameters were selected on the basis of initial sensitivity tests: $mtry = 4$ and $ntree = 5{,}000$. Random forest analyses were implemented in the R package randomForest (Liaw and Wiener 2018).

Model Implementation with Extinct Specimens

We implemented different sets of random forest models; given the missing traits in our incomplete dataset, models needed to be trained once with the missing traits eliminated from the extant ant morphometrics training dataset and once including all traits measured for the complete fossil dataset (fig. 1). For each dataset (complete or incomplete), we implemented three models: using the linear measurements, shape ratios, and log shape variables. Using these three models, we predicted ecological niche aspects of hell ants twice: once using functional morphology, and once using homologous morphology. Thus, each specimen's ecological niche was predicted six times (contingent on whether their measurement set was complete or incomplete): using linear measurements with functional morphology, linear measurements with homologous morphology, shape ratios with functional morphology, shape ratios with homologous

morphology, log shape variables with functional morphology, and log shape variables with homologous morphology. We compiled all model votes into a heat map of model predictions for each specimen with the R package ggplot2 (Wickham 2016) to better visualize the consensus among models for each predicted ecological niche aspect.

Comparative Ecomorphological Niche Occupation

To assess the specificity and breadth of niche occupations in haidomyrmecines, we generated three-dimensional ecomorphological matrices comprising living and extinct predatory taxa. Our taxonomic sampling included five lineages: hell ants and four distantly related extant groups with trap-jaw-like morphology and behavior, wherein workers act as solitary hunters that capture prey, many doing so through rapid closure of specialized mandibles (Hölldobler and Wilson 1990; Gronenberg and Ehmer 1996). While the speed of prey capture is not known in hell ants, haidomyrmecines are united with some extant trap-jaw taxa by the presence of elongate setae (interpreted as trigger hairs) in the path of mandible closure and dramatic morphological adaptations related to predation (Dlussky 1996; Barden and Grimaldi 2012). Importantly, all species within our sampling are primarily solitary hunters (Larabee and Suarez 2014; Barden et al. 2020) in contrast to group raiding or collective prey capture that typify many other predatory ant lineages (Dornhaus and Powell 2010). Trap-jaw mechanisms have evolved at least 10 times in living ants (Larabee and Suarez 2014; Booher et al. 2021); these origins are distributed among four monophyletic lineages. Extant trap-jaw predation has evolved once within each of the subfamilies Ponerinae and Formicinae, thus our sampling included species within relevant genera: *Anochetus* + *Odontomachus* (Ponerinae) and *Myrmoteras* (Formicinae), respectively. There are at least eight trap-jaw origins within the subfamily Myrmicinae, and seven of these have occurred within the genus *Strumigenys*. Our myrmicine sampling therefore included *Strumigenys* as well as the five dacetine trap-jaw genera: *Acanthognathus*, *Daceton*, *Epopostruma*, *Microdaceton*, and *Orectognathus*. Although it is not yet clear whether all dacetine trap-jaws are the product of a single origin, we grouped these taxa in analyses, as they are more closely related to each other than any are to *Strumigenys* (Ward et al. 2015).

To estimate the total number of unique ecomorphological combinations for each specialized predatory lineage, we gathered niche occupation and size data for a total of 982 species, including 15 hell ant species with ecologies estimated under random forest. Our extant sampling represents a minimum of 50% species sampling for each genus. Each species was assigned one of three foraging and three nesting niche aspects according to our modeling results for haidomyrmecines or published natural history obser-

vations for extant taxa (Supplementary Data_Trap Jaw Ecomorphospace in Zenodo [https://doi.org/10.5281/zenodo .7897553]; Sosiak et al. 2023). Because observational data do not exist for all species, we applied published generalizations in some cases (e.g., *Strumigenys* are noted as almost always leaf litter nesting and foraging, thus we assumed this occupation by default except when otherwise noted in the literature). For the predicted hell ant ecomorphological combinations, considering that there were multiple predictions using different models that did not always converge on the same predicted class, we constructed two matrices: an expansive one incorporating all ecomorphological combinations predicted across all models and a conservative second matrix incorporating only predictions using the linear measurements dataset with functional morphology. All niche occupation and size data may be found in Zenodo (https://doi.org/10.5281/zenodo.7897553; Sosiak et al. 2023).

To estimate body size, we gathered minimum and maximum Weber's length (a measurement of mesosoma length and traditional metric of ant size) measurements from taxonomic descriptions and revisions. To include taxa without published morphometric data, we collected Weber's length measurements from publicly available images on AntWeb (AntWeb 2021) using ImageJ version 1.53 (Schneider et al. 2012). We discretized species sizes by delimiting Weber's length ranges for each species into at least one of 12 equal size binnings. Size binning ranges were defined as one-half of the standard deviation of Weber's length measurements across all species. In cases where a species Weber's length range exceeded any one size binning, we assigned multiple size binnings for that species.

We generated three-dimensional ecological disparity values for each lineage following a modification of Chen et al. (2019). We assigned each ecological binning a numerical value from 1 to 3 on the basis of inferred ecological proximity (nesting niche: leaf litter = 1, ground = 2, lignicolous = 3; foraging niche: leaf litter = 1, epigeic = 2, arboreal = 3), while values for the third ecological dimension (body size) were continuous from 1 to 12. We included raw body size, as size can act as a constraint on ecological role in the environment. To reduce the impact of species sampling bias between fossil and extant lineages, we calculated ecological disparity only among unique occupations, not between species. We created a matrix of unique niche occupations for each lineage and calculated intralineage ecological disparity values by summing the distances between niche aspects for all pairwise combinations of unique occupations using the equation. For example, the ecological disparity between unique occupation 1 (uo1) and unique occupation 2 (uo2) would be $|\text{Nesting Niche}_{uo1} - \text{Nesting Niche}_{uo2}| + |\text{Foraging Niche}_{uo1} - \text{Foraging Niche}_{uo2}| + |\text{Body Size}_{uo1} - \text{Body Size}_{uo2}|$. We summarized mean and standard deviations

for each lineage using ggplot2 (Wickham 2016). Visual representations of lineage-specific ecomorphological occupations (ecospaces) were generated using the R package rgl (Adler et al. 2021) and redrawn in Adobe Illustrator. All R analyses were conducted in R version 4.1.0 (R Development Core Team 2021).

Results

Model Performance and Prediction Accuracy

Visualization of extant and extinct morphospace through PCA illustrated that extinct morphospace fully overlaps with extant morphospace representing linear trait measurements and log shape variable measurements and mostly overlaps with extant morphospace representing shape ratio measurements (figs. S3–S5; figs. S1–S8 are available online). Although aspects of hell ant morphology are distinct, hell ant morphospace represented by measurements incorporated in our models is primarily within the bounds of extant diversity. Principal component 1 in the shape ratio morphospace is primarily driven by mandible size relative to body size, which is greater in many hell ants compared with extant ants, likely resulting in the small portion of unique hell ant morphospace. Predictions from shape ratio measurements are often not significantly different from predictions using linear measurements or log shape variable measurements, however, and this along with a great degree of morphospace overlap suggests that extant morphology is an appropriate analog for extinct ecomorphology.

Out-of-bag error rate estimates ranged from 11% to 22%, reflecting accuracies of 78%–89%, depending on the training data partition used and the ecological niche aspect being predicted. While models trained on the linear measurement and log shape variable measurement datasets were typically more accurate than the shape ratio dataset, this was not always the case, and the accuracies were not higher by more than a few percentage points. Consistently, nesting niche and foraging niche were predicted with higher accuracy, while functional role and ecomorph models generally had lower accuracy. Models trained on the complete morphometric dataset were more accurate compared with those trained on the subset morphometric dataset, though the difference was generally only a few percentage points. Additionally, model consensus votes were highest with foraging niche and nesting niche predictions, indicating overall greater confidence in the accuracy of these predictions, while functional role and ecomorph had lower consensus vote totals (fig. 2; tables 3, S4–S51). Trait importance to model accuracies varied somewhat between models, but overall we found eyes, mandibles, legs, antennae, and body size to be essential to model accuracy (figs. S6–S8).

The fossil *Pseudomyrmex macrops* specimen used as a proof of concept was consistently and accurately predicted as a lignicolous arboreal-foraging phytophagivore (fig. 2). The arboreal foraging niche was predicted with the highest confidence, while the phytophagous functional role was predicted with the lowest confidence (fig. 2; tables S4–S27).

Hell ants are primarily recovered as epigeic foragers that nested directly on the ground surface, though several species are predicted as leaf litter nesters and foragers (fig. 2). Additionally, one *Haidomyrmex* morphospecies (*H. sp3*) was partially predicted as a lignicolous nester and arboreal forager, with *Linguamyrmex brevicornis* also partially predicted as an arboreal forager. Hell ants were primarily predicted as predators, both specialist and generalist, though some species were additionally predicted to be omnivorous (fig. 2; tables S4–51). Supporting the general accuracy of our models, we find broad congruence across species when multiple conspecifics were included and also do not recover any strong predictions of unlikely ecological niches: hell ants were not predicted as subterranean nesters, column-raiding foragers, or fungivorous, granivorous, or phytophagous functional roles (fig. 2).

Specimen consensus among the six models used was variable. For some specimens, there was very strong agreement, with all six models predicting the same ecological niche aspect binning; however, there were also cases where two models predicted one niche aspect binning and two predicted another (fig. 2; tables S4–51). There were rarely scenarios where more than two different niche aspect binnings were predicted for a single specimen. More frequently, in cases of split predictions, the shape ratio models or log shape variable models would predict the same aspect binning while the linear measurement models would predict another, as opposed to scenarios where predictions would split along the lines of functional versus homologous morphology used in the testing dataset.

We found no robust differences in niche predictions between specimens measured directly and specimens measured from microCT reconstructions; specimens measured from microCT reconstructions were also predicted as epigeic or leaf litter foragers and nesters (fig. 2; tables S4–51). Additionally, we find that the dealate *Haidomyrmex scimitarus* (measured from a microCT reconstruction) and the worker *H. scimitarus* (measured using light microscopy) were both predicted to be ground-nesting epigeic predators (fig. 2; tables S4–51), illustrating consensus between the two types of input testing data.

Niche Occupation in Extinct and Extant Specialized Predators

Our most conservative estimates of ecological niche occupation suggest that hell ants occupied primarily ground-nesting epigeic niches with some leaf litter occupation, while across-model results recover hell ants within arboreal,
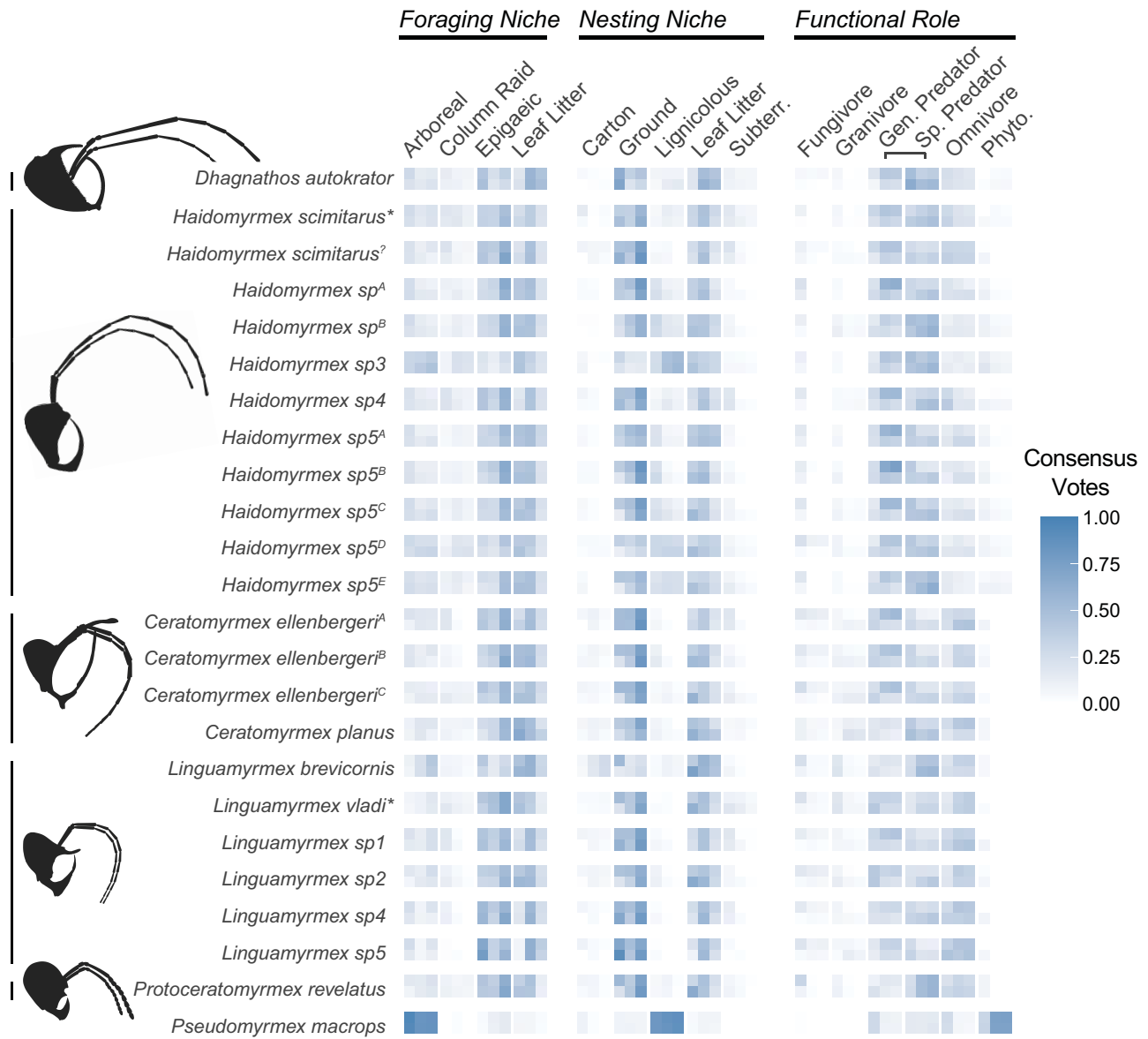
Figure 2: Consensus model votes for niche aspect predictions. Each species' niche aspect predictions are represented by six models derived from alternate datasets (from top left, clockwise): linear functional measurements, log shape variable functional measurements, shape ratio functional measurements, shape ratio homologous measurements, log shape variable homologous measurements, and linear homologous measurements. Taxonomic sampling includes described (named) taxa as well as putative morphospecies. Alternate specimens of the same species are denoted with superscripts. Asterisk denotes specimens included through CT scan reconstruction data. All model votes are available in tables S4–S51; full specimen information is available in table S3.

ground, and leaf litter niches across a moderate body size range. In comparing predicted hell ant ecospace to solitary predator ant ecospace, we find that hell ants occupied at least part of the ecomorphological spaces occupied by each extant trap-jaw lineage (fig. 3). The sister ponerine genera *Anochetus* and *Odontomachus* exhibit the greatest extant ecospace diversity and ecological disparity, occupying most potential ecospace, with species ranging from

~3 mm to ~1.7 cm spanning arboreal, ground, and leaf litter niches (Brown 1978; Hoenle et al. 2020). The most restricted ecospace is occupied by species within the formicine genus *Myrmoteras*, which are minute leaf litter dwellers. The most taxonomically diverse trap-jaw lineage is *Strumigenys* with over 850 species, but the constrained size and primarily leaf litter habits of the genus produce a within-group ecological disparity that is low relative to

Table 3: Out-of-bag accuracies (percentages) for each random forest model constructed

	Nesting niche	Foraging niche	Functional role	Ecomorph
Complete morphometric dataset:				
Linear trait measurements	85.77	83.63	85.88	81.78
Shape ratio measurements	82.92	85.05	80.92	79.84
Log shape variable measurements	88.61	88.97	82.06	82.56
Subset of complete morphometric dataset:				
Linear trait measurements	85.41	82.92	80.53	81.78
Shape ratio measurements	80.07	81.14	82.06	77.91
Log shape variable measurements	86.83	84.34	83.21	81.78

the ponerine lineages with 188 total species (Bolton 2021). Similarly, while total species diversity of hell ants is unknown, even within our limited fossil sample, haidomyrmecines are found to be relatively ecologically disparate and diverse compared with extant lineages.

Discussion

We present here a pipeline for paleoecological niche estimation using machine learning and broad ecomorphological sampling. Because this class of supervised machine learning incorporates nonlinear modeling and has been shown to outperform other discriminant function methods (Pigot et al. 2020; Sosiak and Barden 2021), it represents a powerful tool in the reconstruction of fossil niche occupations. Considerations identified through sensitivity analysis include alternative assumptions of input data, hypotheses of homology, and dataset completeness. Through implementation of this method, we find broad consensus across models for hell ants as leaf litter foraging and nesting or epigeic ground-nesting predators, in contrast to previous assumptions regarding hell ant ecology. Paleoecological niche estimation reveals repeated ecological niche occupation across ant taxa, even across Earth's last major extinction boundary, suggesting functional succession in ant lineages through deep time.

Utility of Random Forest in Paleoecological Estimation

Our results demonstrate the utility of amber specimens in paleoecological estimation and the capacity for microCT reconstructions to aid in data collection. The congruence between the known ecology of extant *Pseudomyrmex* species and the predicted ecology of our sampled fossil *Pseudomyrmex macrops* specimen highlights both that taphonomic preservation of amber specimens does not prohibitively distort morphology and that microCT data from fossil specimens are congruent with morphological data collection derived from extant taxa.

Estimates from our random forest models emphasize the need for selectivity and caution in assembling training data.

We find that accuracy and congruence among our models were influenced more by the input training data rather than the usage of either functional or homologous morphology in testing data; that is, models trained on linear measurement data tended to predict the same nesting or foraging class for hell ants regardless of whether functional or homologous morphology was considered, while models trained on other datasets might predict a different ecology. This trend may reflect variable importance; traits in hell ants that were measured differently on the basis of alternate assumptions of homology were also traits that were not ranked as highly important to model accuracy; therefore, homology assumptions may not have made much difference to the ultimate predicted class. However, this trend may not hold true in other taxa where a questionably homologous trait is of much greater importance to its ecological niche; such cases should be evaluated through assessments of trait importance in model construction. Additionally, we found only a minor loss in model accuracy from models trained on the complete morphometric dataset (17 traits) relative to models trained on the subset morphometric dataset (13 traits). Many of the measurements left out in the subset dataset were ranked as of low importance in the accuracy of the model, highlighting again the necessity of identifying important variables to model accuracy. While the problem of extraneous variables in the model itself is one mostly of unnecessary computing power, it is important to identify crucial traits when sampling fossil data to collate the greatest number of sufficiently complete specimens.

Sources of intraspecific and interspecimen variability in random forest model predictions range from model error to actual ecological variability within the taxa sampled. While we found that each of the six models did not always predict the same ecological niche for one specimen, most frequently these differences were between niche aspects that were physically or ecologically proximate; that is, two models for one specimen might predict generalist predator and specialist predator or leaf litter foraging and epigeic foraging. This variability might reflect morphological similarity among proximate aspects, resulting in model error, but it may also reflect ecological variability within species; for
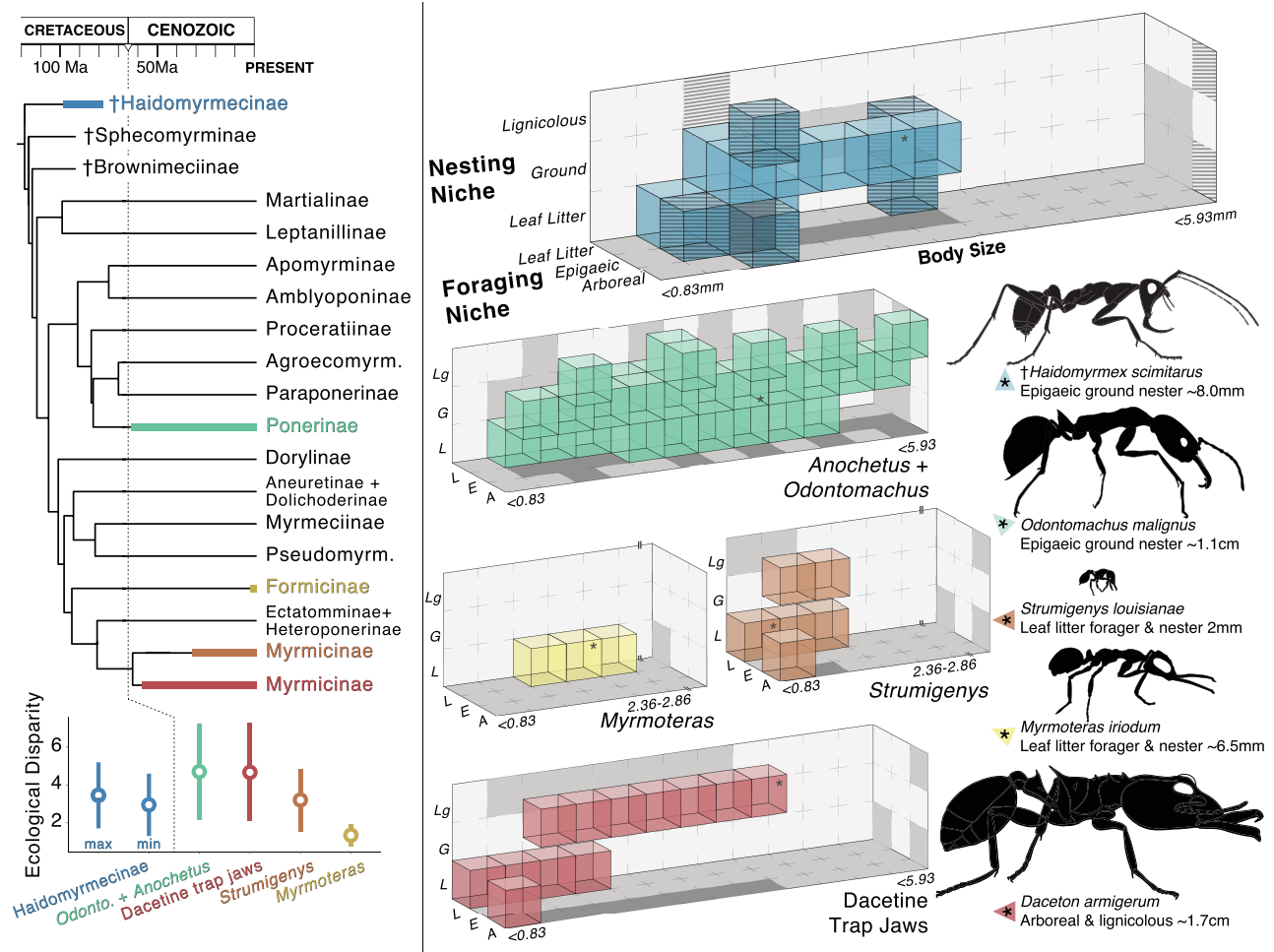
Figure 3: Ecospace occupation of haidomyrmecine and extant specialist predator lineages. *Top left*, subfamily-level time-calibrated phylogeny of ants, with divergence dates from Borowiec et al. (2019). Haidomyrmecinae and extant lineages are denoted by colored bars; haidomyrmecine range is derived from oldest and youngest deposit ages, and extant ranges are based on available crown age estimations for each lineage: Ponerinae: *Anochetus + Odontomachus* (Fernandes et al. 2021); Myrmicinae: *Strumigenys* (Booher et al. 2021); Myrmicinae: dacetine trap-jaws (Ward et al. 2015). No divergence date estimates are available for the formicine trap-jaw genus *Myrmoteras*. *Right*, lineage-specific ecomorphological niche occupations. Each colored cube represents a unique occupied niche. Hashed cubes in haidomyrmecine ecospace indicate maximum hypothetical niche occupation based on all unique combinations estimated across all six random forest models, while remaining cubes reflect only the majority aspect from the linear functional measurement model. All extant ecospaces are compiled from literature. *Bottom left*, within-lineage ecological disparity calculated as average pairwise distance between each unique three-dimensional occupation. Maximum and minimum haidomyrmecine values represent alternate niche occupations described on the right. Ecological disparity values are listed in table S52.

example, predatory ants may supplement their diet with plant material or occasionally forage across strata. While it is difficult to strictly attribute prediction mismatches to model error or ecological variability, we suggest that model error is more readily identifiable when two models predict ecologies with no known affinities. Additionally, consensus votes can be assessed on a specimen-by-specimen basis: when the consensus vote is less than 50%, meaning that the prediction is a result of a plurality of votes rather than a majority, confidence in that prediction is lower. This assessment, however, can be influenced by the number of

classes; in our datasets, prediction accuracy was higher with niche aspects that had fewer classes. It is important to contextualize the number of possible classes with consensus votes.

The implementation of this pipeline requires a taxonomic group with both fossil and extant representatives with morphological continuity that allows for homologous trait measurements across time series. The application of extant trait data in extinct ecological estimation is best suited among lineages that exhibit a high degree of extant diversity relative to fossil samples: the more ecologies

that the extant group occupies, the more likely it is that all potential ecological niches of the extinct group are represented. Predicted ecologies should also be evaluated in the context of other fossil evidence. For example, several extant ant taxa are primarily granivorous seed eaters (Cole 1968; Plowes et al. 2013); thus, the functional role of an extinct species could potentially be predicted as granivorous. However, while grasses first evolved in the early Cretaceous, grassland ecosystems did not develop broadly until later in the Cenozoic, making it unlikely that Mesozoic ants would have been granivorous (Stromberg 2011; Boyce and Lee 2017).

Ecological Extinction and Succession in the Earliest Ants

We recover broad consensus across models for hell ant ecological niche occupation: our models consistently predict haidomyrmecine taxa as leaf litter foraging or epigeic ground-nesting predators, with few outliers (fig. 4). Our results contrast with previous hypotheses suggesting a primarily arboreal lifestyle among hell ants. Initial hypotheses were based on qualitative assessments of morphology (Barden and Grimaldi 2012), expected amber entrapment bias toward arboreal-associated species (Solórzano Kraemer et al. 2015, 2018), and an assertion that hell ants' vertically aligned mandibles might have precluded the fine manipulation of soil required to create ground nests (Dlussky 1996). The susceptibility of arboreal-associated species to extinction during cataclysmic events (Field et al. 2018) also provided a plausible explanation of the hell ants' demise. However, in addition to our own quantitative estimations of hell ant ecology, extant and fossilized behavioral evidence provide additional support for predicted ground and leaf litter habits among haidomyrmecines. Many extant trap-jaw ant species are capable of manipulating soil with their highly specialized mandibles, allowing for ground-nesting trap-jaw species (Cerquera and Tschinkel 2010). Soil nesting is also estimated as the ancestral state among all crown ants, though fossils have not yet been included in such reconstructions (Lucky et al. 2013). Additionally, two fossilized examples of hell ant prey reflect leaf litter and surficial habitats: a beetle larva in association with a *Linguamyrmex vladi* worker (Barden et al. 2017), likely reflecting a humid leaf litter habitat; and a cockroach relative
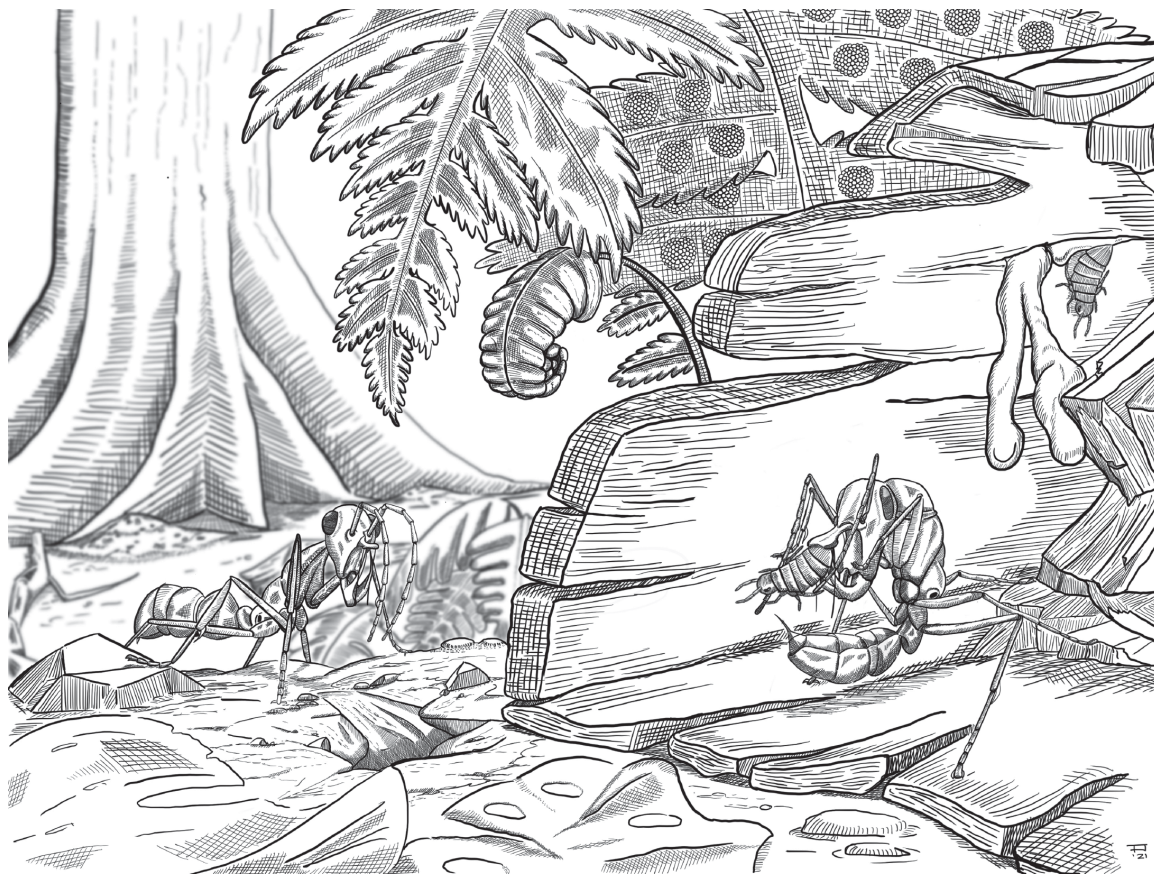

Figure 4: Reconstruction of putative nesting and predatory foraging habits of hell ant *Linguamyrmex vladi*. Artist: John Paul Timonera.

Caputoraptor elegans in association with *Ceratomyrmex ellenbergeri* (Barden et al. 2020), possibly living in leaf litter or surficial strata, although arboreal habits have been proposed (Bai et al. 2018). This reconstructed nesting ecology of hell ants also aligns with proposed extrinsic factors related to the evolution of eusociality (Evans 1977).

While our results do not support the hypothesis of hell ant arboreality as a factor in their extinction, we do find support for specialized predation in several hell ant genera. Species with specialized diets are at higher risk of extinction during cataclysmic events because of the greater likelihood of their food sources' extinction and lack of flexibility in diet (Chichorro et al. 2022; Machado et al. 2022). While it is unclear whether haidomyrmecines became extinct prior to the Cretaceous-Paleogene boundary or were lost during the mass extinction event, a specialized predatory diet may have been a factor in their demise.

Our comparison of ecomorphospace occupation in hell ants and in four monophyletic lineages with independent origins of trap-jaw mechanisms illustrates ecological coherence across deep time and distantly related lineages. Our reconstructions suggest that hell ants were functional analogs to many modern-day trap-jaw lineages in surficial and leaf litter arthropod communities: solitary-foraging hunters seeking out prey across the forest floor and in interstitial leaf litter spaces. The morphological adaptations of modern-day trap-jaw workers necessitate solo foraging: rapid power-amplified closure of their specialized mandibles following the activation of elongate trigger setae in the path of mandible movement, subsequently stinging their prey. This specialized prey capture typically precludes group predation; workers individually subdue a single prey item before returning to the nest (Beckers et al. 1989; Larabee and Suarez 2014). There are some morphological traits that support a trap-jaw mechanism in hell ants, including trigger hairs and a structurally reinforced clypeal paddle at the point of mandible articulation (Barden and Grimaldi 2012; Barden et al. 2017). Moreover, there is direct paleoethological evidence of solitary foraging among hell ant workers (Barden et al. 2020).

Our paleoecological estimation allows for the recovery of repeated ecological niche occupation across lineages of predators. Hell ants occupied approximately 10% of hypothetical potential sampled ecomorphospace and yet radiated into leaf litter, surficial, and, to a lesser degree, arboreal habitats in an ecomorphospace occupation that mirrors living lineages; hell ants entirely or at least partially overlap with each independent origin of extant trap-jaw ants. Even as molecular-based divergence estimates place the origin of modern ants during the Cretaceous (Moreau et al. 2006; Borowiec et al. 2019), the earliest extant trap-jaw predators emerged later in the Cenozoic, from ~65 Ma in ponerines to ~35 Ma in *Strumigenys*, and after

the last appearance of hell ants in the fossil record (fig. 3; Ward et al. 2015; Booher et al. 2021; Fernandes et al. 2021). The last known hell ant fossil dates to 78 Ma in Campanian-age Canadian amber (McKellar et al. 2013). It is unclear precisely when hell ants became extinct, but the overlap of ecospace occupation between hell ants and the ponerine and dacetine lineages arising very soon after the Cretaceous-Paleogene extinction may be a signature of faunal turnover in niche occupation. The extinction of hell ant lineages may have provided vacant ecospace that was filled by modern trap-jaw lineages. Additionally, while we included most known trap-jaw species in our extant ecospace reconstructions, fossil sampling remains much more limited; thus, the full ecospace occupation of hell ants was probably broader than our current reconstruction suggests. The ecological breadth of the more than 1,000 modern trap-jaw ant species (Bolton 2021) may represent echoes of their Cretaceous counterparts.

Faunal and ecological turnovers are a frequent feature of evolutionary history on Earth; the fossil record contains a plethora of examples (Sallan et al. 2011; Benson and Druckenmiller 2014; Moon and Stubbs 2020). We provide here a new quantitative framework for testing a variety of paleoecological hypotheses, including evaluations of ecology-based extinction risk, ecological succession in deep time, and competition between temporally and spatially proximate lineages. Our investigation of hell ant ecology generated a new test of ecology-linked extinction and revealed a detailed ecological turnover in ecomorphospace occupation among temporally disjunct monophyletic lineages. By reconstructing the ecological community of the earliest ants, we find repeated lineage occupation of ecospace that is consistent with functional succession across Earth's last mass extinction event.

pursuant to the proposed boycott of Burmese amber by the Society of Vertebrate Paleontologists (Rayfield et al. 2020). Some specimens are located in a private collection, while others are located in institutional museums: specimen repositories are indicated in the supplemental PDF associated with this article, and we have provided photomicrographs of all specimens residing in a private collection. All data associated with this article are available in the supplemental PDF, including all morphometric measurements for privately owned specimens.

Statement of Authorship

C.S. and P.B. conceptualized the study; C.S. and P.B. developed methodology and conducted statistical analyses; C.S., T.J., V.P., J.P.T., and P.B. all contributed to data compilation and collection, data visualization, writing of the original draft, and reviews and edits of the final draft.

Data and Code Availability

All data and scripts needed to reproduce the analyses and evaluate the conclusions in the paper are available in the paper, supplemental PDF, and Zenodo (https://doi.org/10.5281/zenodo.7897553; Sosiak et al. 2023).

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