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Evolution of the latitudinal diversity gradient in the hyperdiverse ant genus *Pheidole*

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Abstract

Aim: The latitudinal diversity gradient is the dominant geographic pattern of life on Earth, but a consensus understanding of its origins has remained elusive. The analysis of recently diverged, hyper-rich invertebrate groups provides an opportunity to investigate latitudinal patterns with the statistical power of large trees while minimizing potentially confounding variation in ecology and history. Here, we synthesize global phylogenetic and macroecological data on a hyperdiverse (> 1,100 species) ant radiation, *Pheidole* and test predictions of three general explanations for the latitudinal gradient: variation in diversification rates, tropical conservatism and ecological regulation.

Location: Global.

Time period: The past 35 million years.

Major taxa studied: The hyperdiverse ant genus *Pheidole* Westwood.

Methods: We assembled geographic data for 1,499 species and morphospecies, and inferred a dated phylogeny for 449 species of *Pheidole*, including 167 species newly sequenced for this study. We tested for correlations between diversification rate and latitude with Bayesian analysis of macroevolutionary mixtures (BAMM), hidden state speciation and extinction (HiSSE), geographic state speciation and extinction (GeoSSE), and a non-parametric method (FiSSE), evaluated evidence for richness steady state, and examined patterns of diversification as *Pheidole* spread around the globe.

Results: There was no evidence of systematic variation of net diversification rates with latitude across any of the methods. We found that *Pheidole* diversification occurred in bursts when new continents were colonized, followed by a slowdown in each region, but there is no evidence richness has saturated at an equilibrium in any region. Additionally, we found latitudinal affinity is moderately conserved with a Neotropical ancestor and simulations show that phylogenetic inertia alone is sufficient to produce the gradient pattern.

Main conclusions: Our results provide no evidence that diversification rates vary systematically with latitude. Richness is far from steady state in each region, contrary to the ecological regulation hypothesis, although there is evidence that ecological

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opportunity promotes diversification after colonization of new areas. The fact that niche conservatism is strong enough to produce the gradient pattern is in accord with the tropical conservatism hypothesis. Overall, these results shed light on the mechanisms underlying the emergence of the diversity gradient within the past 34 million years, complementing recent work on deeper time-scales, and more generally contribute toward much-needed invertebrate perspective on global biodiversity dynamics.

KEYWORDS
ants, diversification rate, diversity regulation, latitudinal diversity gradient, macroevolution, tropical conservatism

1 | INTRODUCTION

Understanding how ecological and evolutionary processes interact with historical factors to shape global biodiversity patterns remains a major goal of biology. The latitudinal diversity gradient (LDG) is the most general biogeographic pattern, yet we still lack a consensus understanding of its mechanisms (Fine, 2015; Mittelbach et al., 2007; Pianka, 1966; Willig, Kaufman, & Stevens, 2003). This is likely because many biological, physical and historical factors that could plausibly affect diversity vary systematically with latitude, and thus a large number of hypotheses have been developed to explain the pattern. However, testing the predictions of different hypotheses empirically and evaluating their relative merits has proven to be a challenge.

Recently, the synthesis of large-scale geographic datasets along with large-scale phylogenetic data has provided new opportunities for empirical evaluation of hypotheses for the mechanisms underlying the LDG. These tests have mainly focused on vertebrates (e.g. Cardillo, Orme, & Owens, 2005; Duchêne & Cardillo, 2015; Jetz, Thomas, Joy, Hartmann, & Mooers, 2012; Pigot, Tobias, & Jetz, 2016; Pyron & Wiens, 2013; Rolland, Condamine, Jiguet, & Morlon, 2014; Siqueira, Oliveira-Santos, Cowman, Floeter, & Algar, 2016; Weir & Schluter, 2007) and woody plants (Kerkhoff, Moriarty, & Weiser, 2014), as those are the taxa with large-scale comprehensive data available. Several pioneering studies have examined latitudinal diversification patterns in insects (e.g. Condamine, Sperling, Wahlberg, Rasplus, & Kergoat, 2012; McKenna & Farrell, 2006; Moreau & Bell, 2013; Hawkins & DeVries, 2009; Owens et al., 2017; Pie, 2016), although data-deficiency of most invertebrate groups makes taxonomic and/or geographic scope a challenge for analysis.

Among invertebrates, ants are emerging as an exemplar taxon for global biodiversity studies. Ants are ecologically dominant in most terrestrial ecosystems and are, for an insect group, relatively well documented scientifically. Moreover, their diversity is high, but not intractably so, with richness on the same order as major vertebrate groups (~15,000 described ant species), and ants exhibit a marked LDG (Dunn et al., 2009; Kaspari, Ward, & Yuan, 2004). Recently, a new comprehensive dataset has been compiled that gives the known geographic distribution of all described ant species across > 400 geographic regions around the globe (Guénard, Weiser, Gomez, Narula, & Economo, 2017). These data, combined with progress toward reconstructing the ant tree of life (Brady, Schultz, Fisher, & Ward, 2006; Moreau & Bell, 2013; Moreau, Bell, Vila, Archibald, & Pierce, 2006; Ward, Brady, Fisher, & Schultz, 2015), allow for inferences of the evolutionary underpinnings of large-scale diversity patterns in ants.

Here, we use the globally distributed, hyperdiverse (> 1,100 described species) ant genus *Pheidole* as a model taxon to test hypotheses for the LDG. While over a hundred hypotheses have been proposed to explain the gradient (Fine, 2015; Mittelbach et al., 2007; Willig et al., 2003), these can be placed under three umbrella hypotheses: (a) the diversification rate hypothesis (DRH), (b) the tropical conservatism hypothesis (TCH) and (c) the ecological regulation hypothesis (ERH).

The DRH posits that there is some causal factor that affects speciation and/or extinction rates and varies with latitude (e.g. reviewed in Fine, 2015; Mittelbach et al., 2007; Pianka, 1966). This leads to a latitudinal disparity in species accumulation rate that underlies the gradient, rather than any regulation of total species numbers. Many such potential causal factors have been proposed. For example, temperature may affect mutation rates, which in turn could affect the rates of evolution of reproductive incompatibilities (Rohde, 1992). Or, extinction rates could be higher in the temperate than tropical zone to due greater climatic variability (Rolland et al., 2014; Weir & Schluter, 2007). The prediction of the DRH is straightforward: net diversification rate inferred from a phylogeny should be higher in tropical lineages compared with extratropical lineages.

The TCH (Pianka, 1966; Wiens & Donoghue, 2004) posits that the relative youth of colder temperate biomes combined with the inertia of niche evolution (phylogenetic niche conservatism: Losos, 2008; Wiens & Graham, 2005) has limited the accumulation of diversity in the temperate zone. In this scenario, net diversification rates or equilibrium levels do not necessarily vary with latitude, and the difference in richness is mainly due to time for diversification (Stephens & Wiens, 2003). This idea is based on the fact that historically the Earth has been much warmer than it is now, and much of what is now the temperate zone was covered by "megathermal" biomes. This hypothesis is supported by the fossil record; many lineages that used to occur in the Palaearctic are now limited to tropical latitudes. This is true for ants as well; the Baltic amber ant fauna

from the late Eocene has greater affinity to modern Indo-Australian faunas than modern Palaearctic faunas (Guénard, Perrichot, & Economo, 2015). The main prediction of this hypothesis is that the ancestral region of most groups is the tropics (or areas with what we recognize now as "tropical" climates that may have previously extended out of the tropics). Furthermore, transitions out of the tropical zone are rare, and thus the temperate clades are younger and nested within tropical clades. Transitions from tropical to temperate zones should be difficult because of the many nontrivial adaptations that ectothermic organisms need to survive at higher latitudes. An additional prediction of the TCH is that the accumulation of lineages in the temperate zone through dispersal or cladogenesis has mostly occurred after the Oligocene cooling, 34 Ma.

The ERH posits that, due to causal factors that vary with latitude, more species can coexist locally and regionally in tropical ecosystems than in temperate ecosystems. In this case, diversity is saturated at or near an ecological limit, and this "carrying capacity" of species varies with latitude. Equilibrium diversity levels may be regulated by factors such as productivity, perhaps mediated through competition and limiting similarity (Hurlbert & Stegen, 2014b; Pianka, 1966; Rabosky & Hurlbert, 2015). Speciation and extinction rates may vary over time to regulate richness at the requisite equilibrium level for a geographic region, but latitudinal factors acting on speciation/extinction are not causally responsible for the disparity in diversity. Likewise, latitudinal affinity may be highly conserved or evolve quickly, but it would be immaterial to the origins of the gradient if diversity is saturated and regulated at levels that vary with latitude.

These hypotheses have been tested across broad taxonomic scales (e.g. birds: Jetz et al., 2012, amphibians: Pyron & Wiens 2013; mammals: Buckley & Jetz, 2007; Rolland et al., 2014), and predictions of the DRH and TCH have been recently examined on the scale of all ants using a comprehensive geographic dataset (Economo, Narula, Friedman, Weiser, & Guénard, 2018; also see Moreau & Bell, 2013; Pie, 2016). That study found that tropical lineages are more ancient than extratropical lineages, which mainly arose since the Oligocene cooling (past 34 Myr), consistent with the TCH. Further, they found that diversification rate was highly heterogeneous but uncorrelated with latitude among ant clades, inconsistent with the DRH. Due to the limitations of phylogenetic data at such broad taxonomic and time scales, they could not test for ecological regulation (ERH). As with other studies on broad taxonomic scales, the analyses across all ants provided the statistical advantages of big datasets and a deep-time perspective. However, deep-time analyses also can be confounded by the fact that many ecological, functional trait, and historical factors may affect macroevolutionary rates in ways that could obscure underlying latitudinal effects. For example, ant diversification rates have been shown to be heterogeneous across clades (Moreau & Bell, 2013; Pie & Tschá, 2009) and possibly related to functional traits (Blanchard & Moreau, 2017). Analyses of closely related lineages allow us to control to some extent for differences in biology unrelated to the variable of interest (in this case latitudinal affinity), while the large trees of hyperdiverse radiations enhance statistical power to detect trait-dependent diversification (Gamisch, 2016). Moreover, latitudinal gradients are often present within individual clades that evolved recently (Economo, Klimov, et al., 2015), and different ecological and evolutionary dynamics can dominate at different phylogenetic scales (Graham, Storch, & Machac, 2018). Studies across broad phylogenetic/time scales may miss the relevant scale of variation in diversification rate. Thus, the analysis of closely related lineages within younger, hyper-rich radiations provides an important complement to studies of larger taxa and longer time-scales.

The global radiation of *Pheidole* arose entirely since the Oligocene cooling (last 34 Myr), during which time it has evolved a latitudinal gradient echoing the pattern for all ants (Economo, Klimov, et al., 2015). Thus, *Pheidole* presents an opportunity to examine diversification dynamics in this most recent period since the Oligocene, a period when many ant lineages transitioned out of the tropics. While the low number of older extratropical ant lineages is consistent with the TCH (Economo et al., 2018), there is still an open question of whether niche conservatism, diversification rate differences, or ecological saturation explain the emergence of the gradient since the Oligocene. According to the TCH, the tropical ancestry of *Pheidole* combined with a low rate of evolution in latitudinal affinity (i.e. high phylogenetic niche conservatism) explains why there are more species in the tropics in the absence of latitudinal differences in macroevolutionary rates or regulation at different levels. The DRH predicts that *Pheidole* is diversifying more rapidly in the tropics, and this explains the current observed disparity in richness. The ERH posits that diversity is saturated at different levels across latitudes, and thus we should see a period of high net diversification rates early in the radiation followed by declines to near zero as richness reaches steady state or "carrying capacity". The ERH is thus an equilibrium hypothesis while the DRH and TCH are both non-equilibrium hypotheses.

Here, we reconstruct a new global *Pheidole* phylogeny—the most comprehensive to date—increasing substantially the taxonomic and geographic coverage from previous studies of the genus (Economo, Klimov, et al., 2015; Economo, Sarnat, et al., 2015; Moreau, 2008; Sarnat & Moreau, 2011). We use the new phylogeny and geographic data from the Global Ant Biodiversity Informatics (GABI) database to test predictions of the three umbrella hypotheses for the latitudinal gradient. As mechanisms involved with different hypotheses can be simultaneously operating (for example, speciation rate can vary with latitude even while niche conservatism limits colonization of the extratropics), our goal is to rule out mechanisms rather than isolate a single exclusive answer. The analysis of this famously hyperdiverse radiation will advance our general understanding of the latitudinal gradient, the most pervasive pattern of life on Earth.

2 | METHODS

2.1 | Geographic data

Our geographic data are based primarily on the GABI Project database (Guénard et al., 2017), which can be viewed through the

website antmaps.org (Janicki, Narula, Ziegler, Guenard, & Economo, 2016), and secondarily on the personal collection records of the authors (all of which are available on AntWeb.org). The former focuses on described species, while the second was used to supplement data on morphospecies for taxa included in the phylogenetic analysis. Because many records of ant occurrence are not associated with geocoordinates, we assigned each record to a system of 415 polygons around the world (Figure 1). Latitudinal range for a species was estimated as minimum and maximum latitudes over all polygons in which a species occurs, excluding occurrences where the species is considered exotic and dubious records. For statistical analyses, we used either the absolute midpoint latitude of the range or an index of tropicality (fraction of latitudinal range in the tropics—fraction of latitudinal range outside the tropics, with ± 23.5° latitude as the boundary of the tropics). For tests using a binary coding of latitudinal state, we separated tropical and extratropical taxa based on midpoint latitude below or above 23.5°.

3 | PHYLOGENY RECONSTRUCTION

3.1 | Taxon selection

Compared with many other large ant radiations, the effort to reconstruct the phylogenetic history of *Pheidole* is relatively far along. A series of studies, beginning with Moreau (2008) and followed by others (Economo, Klimov, et al., 2015; Economo, Sarnat, et al., 2015; Sarnat & Moreau, 2011) has produced a broad picture of the evolutionary history of the genus. However, for the purposes of understanding geographic patterns of diversification, having a larger and more proportionally sampled phylogeny will provide additional statistical power and more robust results. Thus, we continued sampling *Pheidole* taxa for sequencing, focusing on sampling more taxa from the Neotropics, Madagascar and Southeast Asia, which had been undersampled in previous analyses. In all, we increased the number of species from 282 taxa in the most recent global *Pheidole* phylogeny (Economo, Klimov, et al., 2015) to 449 taxa in the current contribution (Supporting Information Table S1).

3.1.1 | Estimation of sampling completeness

One source of uncertainty in large-scale analyses of diversity is bias in taxonomic completeness overall and among different areas, particularly in relatively poorly known groups such as insects. While there is still a pronounced latitudinal gradient in *Pheidole* even among described species, there are undoubtedly many undescribed species in the genus, and it is probable they are disproportionately found in the tropics. While accounting for unobserved species is a challenge in any analysis, we devised an approximate method to calculate sampling completeness across areas given the information available, and use these estimates in our analysis of diversification rate. The details of our calculation are in Appendix S1, Supporting Information.

3.1.2 | DNA sequencing

Previous molecular work (Economo, Klimov, et al., 2015; Moreau, 2008; Sarnat & Moreau, 2011) on *Pheidole* has generated a dataset based on eight nuclear loci [histone H3.3B F1 copy (His3.3B), long wavelength sensitive opsin 1 (Lop1), glutamate receptor ionotropic, kainate 2-like (GRIK2), unc-4 homeodomain gene (unc_4), uncharacterized locus LOC15 (LOC15), carbomoylphosphate synthase (CAD), elongation factor 1-alpha F2 (EF-1α F2), DNA topoisomerase 1 (Top1)] and one mitochondrial locus [cytochrome oxidase 1 (CO1)]. In a previous study (Economo, Klimov, et al., 2015), all nine loci were sequenced for a subset of 65 taxa representing the main *Pheidole* lineages around the world, while three loci (COI, Lop1 and His3.3B) were sequenced for all taxa to fill out the clades (217 taxa). This hierarchically redundant sampling design was chosen for reasons of cost and time efficiency and to maximize the number of taxa, combined with the fact that many of the slow-evolving nuclear genes provide less information on recent divergences.

We added 167 new *Pheidole* taxa to this existing dataset by extending this sampling design and sequencing COI, Lop1 and His3.3B. We did not plan to sequence all nine loci unless we found

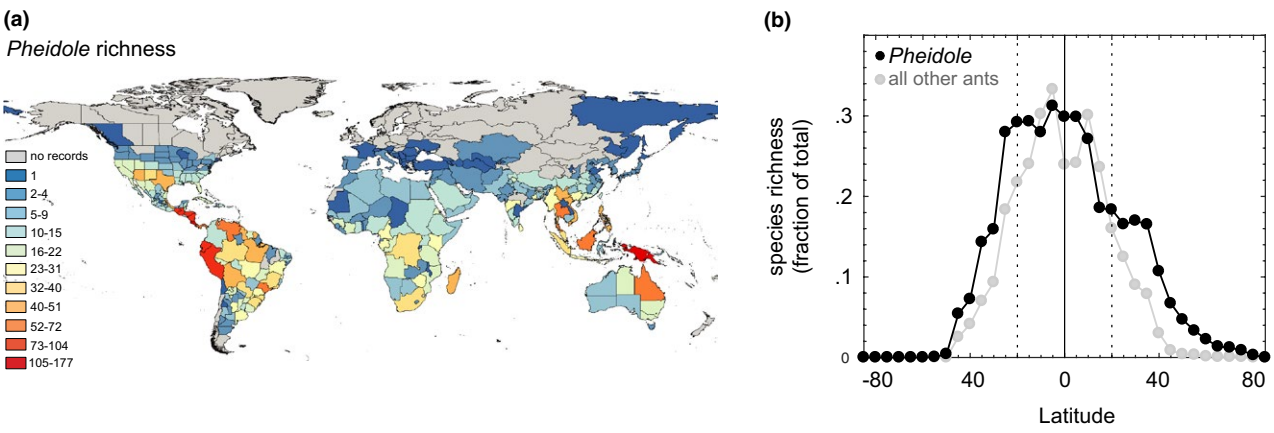

FIGURE 1 Global patterns of *Pheidole* species richness plotted by (a) geographic region and (b) 5-degree latitudinal band for 1,138 described species/subspecies and 361 morphospecies. For comparison, latitudinal distributions of 13,771 ant species excluding *Pheidole* are also depicted. Latitudinal richness is expressed as fraction of total richness (1,499 for *Pheidole*, 13,771 for all other ants)

novel divergent clades not represented by taxa with all nine genes sequenced in the earlier study (and we did not). Ant samples from field collections fixed in 95% EtOH were extracted for DNAs using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The whole ant body was incubated in the extraction buffer without grinding during the first step, and then the complete ant specimen was removed before filtering and cleaning the extracts via a provided column. Extracted DNAs were subsequently used for PCR reactions for one mitochondrial (CO1; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) and two nuclear (His3.3B and Lop1) regions. Each reaction contained 0.5 μl of extracted DNA, 1 μl of 10 × buffer, 0.75 μl of $MgCl_2$, 0.5 μl of 10 mN dNTPs, 0.2 μl of 1% Bovine Serum Albumin, 0.4 μl of each primer, 0.04 μl of Taq DNA polymerase (Invitrogen, Carlsbad, CA) and double-distilled H_2O (ddH_2O) to make a 10 μl reaction in total. Standard PCR procedures were employed with annealing temperatures of 52, 60 and 60 C for CO1, His3.3B and Lop1 regions, respectively. The amplicons were sequenced via an ABI^{3700} machine (Applied Biosystems, Foster City, CA) by the Sequencing Core at the University of Michigan. Sequences were checked using SeqMan (DNAStar Inc., Madison, WI).

3.1.3 | Phylogenetic tree inference

We used Bayesian methods to infer a dated *Pheidole* phylogeny including 449 ingroup taxa (Supporting Information Table S2). To generate codon-aware alignments for these loci, we first searched National Center for Biotechnology Information (NCBI) non-redundant Conserved Domain Search (CDS) database (Clark, Karsch-Mizrachi, Lipman, Ostell, & Sayers, 2016) for reliable amino acid sequences for all loci and retrieved such sequences for seven of the nine loci with the following accession numbers: AIM2284.1 (CAD), ABW70333.1 (CO1), EZA53539.1 (EF-1α F2), EGI60526.1 (His3.3B), ABW36758.1 (Lop1), EGI59282.1 (unc-4) and AIM43286.1 (Top1). These sequences were used as references for generating codon-aware alignments. The CAD, unc-4 and Top1 alignments generated using MAFFT v. 7.205 (Katoh & Standley, 2013) (–retree 4; –maxiterate 1,000) showed no frameshift mutations and/or insertions and deletions. However, the CO1, EF-1α F2, His3.3B and Lop1 alignments did not match the reference sequences, showing disruptions in the translated amino acid alignments (such as the presence of numerous stop codons). For these loci, we used a codon-aware alignment software, MACSE v. 1.01b (Ranwez, Harispe, Delsuc, & Douzery, 2011), to generate the alignments. Reverse translations of the reliable amino acid reference sequences, accounting for all possibilities at each codon position, were passed as reliable input sequences to the software; we were able to assign codon positions within the exons in these seven loci. The resulting alignments were manually inspected and cleaned using GENEIOUS R8 software Kearse et al. (2012). Furthermore, we identified, extracted, and separately aligned intronic regions wherever necessary. The remaining two loci, LOC15 and GRIK-2, were aligned using MAFFT. We concatenated all nine alignments and once again manually cleaned the master alignment, resulting in an alignment containing 8,839 sites.

We used PARTITIONFINDER v. 1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012) to determine the partitioning scheme and corresponding models of molecular evolution. The model scope included HKY, HKY+Γ, SYM, SYM+Γ, GTR, GTR+Γ, TrN, TrN+Γ, K80, K80+Γ, TrNef, TrNef+Γ, JC and JC+Γ, branch lengths were set to "linked", and model selection and comparison was set to Bayesian information criterion (BIC). PARTITIONFINDER identified an optimal scheme containing 16 partitions (Supporting Information Table S3). We used CLOCKSTAR (Duchêne, Molak, & Ho, 2014) to determine the optimal number of clock models across our partitions for relaxed-clock phylogenetics, and a single linked clock was preferred based on the SEMmax criterion.

Our primary phylogenetic inference was conducted in BEAST2 v. 2.1.3 (Bouckaert et al., 2014), but we first performed maximum likelihood (ML) reconstruction in RAxML v. 8.0.25 (Stamatakis, 2014). Using the partitioning scheme described above and the GTR+Γ model, we ran 75 ML inferences with 1,000 bootstraps to find the ML tree. Using the *chronos* function in the *ape* package in R (Paradis, Claude, & Strimmer, 2004; R Core Team, 2018), we scaled the tree by calibrating the root node to a range of 50–60 Myr. This tree was used as the starting tree for the BEAST2 analyses, but the topology was not fixed.

Unfortunately, there are no reliable fossil calibrations available to date nodes *within* the genus. Thus, the age of the group can only be informed by the age of the stem node and information from fossils in related taxa across the subfamily Myrmicinae. Because our analysis is concentrated within *Pheidole*, we preferred to use the stem node age distribution (i.e. the most recent common ancestor of *Pheidole* and its sister lineage *Cephalotes* + *Procryptocerus*) inferred as in a much larger analysis of the subfamily Myrmicinae (Ward et al., 2015) that could make use of a broad range of molecular and fossil data. Following those results, the stem node calibration was set to a normal distribution (mean: 58.0 Mya, sigma, 4.8 Myr). Further analysis of the *Pheidole* fossil record with a goal to place fossil taxa within the *Pheidole* phylogeny and refine dating of different nodes in the tree represents an important need for future phylogenetic work on the genus. Despite this limitation, the analyses in this paper depend mostly on relative—rather than absolute—ages, and we draw no conclusions based on the precise timing of nodes in the tree.

We used a relaxed lognormal clock model linked across partitions (due to the CLOCKSTAR results), and used the partitioning scheme and models identified with PARTITIONFINDER. Six independent analyses were run and chains were stopped between 45 and 80 million generations, after we observed convergence using TRACER software v. 1.6.0 (Rambaut 2014). We discarded the leading 33% of saved states as burnin, combined the remaining trees from all runs to create the posterior set, and generated the maximum clade credibility tree and nodes set to median height. After pruning the outgroup, this tree was used for all subsequent analyses.

3.2 | Macroevolutionary rate inference

We took several complementary approaches to estimating macroevolutionary rates and potential dependencies on latitude, primarily basing

our analysis on Bayesian Analysis of Macroevolutionary Mixtures (BAMM; Rabosky, 2014), Hidden State Speciation and Extinction (HiSSE; Beaulieu & O'Meara, 2016) and Fast, Intuitive State-Dependent Speciation-Extinction analysis (FiSSE; Rabosky & Goldberg, 2017), with a secondary analysis using Geographic State Speciation and Extinction (GeoSSE; Goldberg, Lancaster, & Ree, 2011). These methods each have their strengths and weaknesses and thus our approach was to use them collectively to seek conclusions about our data that are robust to methodological assumptions and implementation.

The main advantage of BAMM is that complex mixture models can be assessed with rate shifts across the tree, including potentially accelerating and/or decelerating diversification rates. While trait-dependent diversification models are not fit directly, trait-diversification correlations can be assessed post hoc using structured rate permutations that estimate correlations while accounting for phylogenetic dependency (Rabosky, Title, & Huang, 2015). We use BAMM to test for correlations between latitude and net diversification rate, and evaluate evidence of decelerating diversification to a steady state (ecological regulation of diversity) overall and in relation to the colonization of continents.

While BAMM has strengths in inferring complex mixtures of diversification processes, it is not explicitly trait-dependent, and the State-dependent Speciation and Extinction (SSE) family of methods explicitly fits models of trait-dependent diversification. SSE methods have been developed with different kinds of trait data, either based on binary traits [Binary State Speciation and Extinction model (BiSSE); Maddison, Midford, & Otto, 2007], continuous traits (Quantitative State Speciation and Extinction (QuaSSE), FitzJohn, 2010) or explicitly geographic traits (Geographic State Speciation and Extinction (GeoSSE), Goldberg et al., 2011). While these methods are explicitly for inferring trait-dependent speciation and extinction, they have the problem that differences in the focal trait are the only mechanisms that can cause shifts in macroevolutionary rates. If the real process has complex rate shifts then a more complex trait-dependent model may fit better than a homogeneous null model, even if the shifts are not related to the traits per se, leading to Type-I errors (Rabosky & Goldberg, 2015). These problems are at least partially solved by HiSSE (Beaulieu & O'Meara, 2016), a method that fits binary trait-dependent speciation and extinction models that can be formally tested against similarly complex trait-independent models. We thus primarily used HiSSE for our analysis. As GeoSSE has been implemented for explicitly geographic dynamics, we also fit that model as a secondary test and present that analysis in the Appendix S2, Supporting Information.

Finally, as an additional test for variation in speciation rate with latitude, we used a nonparametric method, FiSSE (Rabosky & Goldberg, 2017), that does not depend on an assumed model structure and is robust to false inferences of trait-dependent evolution given a range of underlying complex evolutionary dynamics. FiSSE is limited to testing speciation rate differences; it does not directly test for net diversification rate differences. However, many (but not all) hypotheses for why diversification rate could vary with latitude are based on mechanisms acting on speciation rate, so it is a partial test of the broader DRH.

3.2.1 | BAMM implementation

We estimated net diversification, speciation and extinction rates through time for the inferred *Pheidole* tree using the program BAMM v. 2.5 (Rabosky 2014). The initial values for speciation rate, rate shift and extinction rate were estimated using the setBAMMpriors function from the R package BAMMtools (Rabosky et al., 2014). Specifically, a total of 2×10^8 generations of rjMCMC searches with samples stored every 8,000 generations were performed, and a total of 1,000 post-burnin samples (50%) were retained. We performed two BAMM runs for each of three assumptions about sampling completeness (Low, Medium, or High estimate, henceforth: L, M, H) accounted for by changing the GlobalSamplingFraction parameter (.3, .22 and .18, respectively, see Appendix S1, Supporting Information for justification). To account for potential oversampling of Nearctic species, we performed a series of runs where we lowered the number of Nearctic species by randomly pruning 21 (of total 48) Nearctic tips from the tree 10 times and performed a BAMM run on each replicate, using the M assumption for the GlobalSamplingFraction parameter.

Using the posteriors generated from these Markov Chain Monte Carlo (MCMC) runs, we sought to (a) explore the overall pattern of *Pheidole* diversification, (b) assess whether there is evidence of diversity regulation, particularly decelerating diversification to zero over time and after colonization of new areas, and (c) test for latitudinal dependency in diversification rate while accounting for phylogenetic non-independence. We visualized the lineage-specific diversification with the plot.bammdata function from BAMMtools, and the time plot of clade-specific diversification rate was plotted with the plotRateThroughTime function. We used the Structured Rate Permutations on Phylogenies (STRAPP; Rabosky & Huang 2015) method to test for significance of any latitude-diversification correlations. We tested for diversification rate versus either tropicality index or absolute midpoint latitude (one-tailed, 10,000 iterations, Spearman's rho as test statistic). We also checked whether our results were robust to using Pearson correlation as test statistic or coding latitude as a binary variable (tropicality > 0 or tropicality < 0) and using a Mann–Whitney *U*-test.

3.2.2 | HiSSE implementation

The HiSSE approach (Beaulieu & O'Meara, 2016) extends the BiSSE (Maddison et al., 2007) framework with two advances. First, the HiSSE model itself allows for more complex models in which macroevolutionary rates can be the function of the focal trait and a hidden state. Thus, if our focal character has states 1 and 0 (in our case tropical and extratropical), there could be an influence of a second unobserved character (with states A and B) on a macroevolutionary rates λ and μ (λ_{0A}, λ_{0B}, λ_{1A}, λ_{1B}). Second, importantly, it allows the fitting of null character-independent models (CID) in which a hidden factor(s) underlies diversification rate changes without the influence of the focal trait under investigation. This allows trait-dependent BiSSE models to be compared to a CID of similar complexity (CID-2, with

two hidden states A and B) and more complicated HiSSE models to be compared to models of similar complexity (CID-4, with four hidden states A, B, C, D). BiSSE (trait dependent speciation-extinction), HiSSE (trait-dependent speciation/extinction with hidden states that also affect speciation/extinction) and CID (trait-independent models with hidden states that affect speciation/extinction) are best used together and models with all structures can be compared.

We fit a range of models with increasing complexity, starting with the BiSSE family of models under the following sets of constraints on the parameters: all diversification and transition rates equal among states, diversification equal but transition rates different (i.e. speciation and/or extinction changes with latitude, but transition rates among temperate and tropical are equal), diversification different but transition rates equal (i.e. speciation and extinction vary with state, but transition rates are equal), or all rates free and unconstrained to vary with state (the full BiSSE model).

The HiSSE models allow speciation/extinction/transition rates to vary with the focal trait and also with two hidden traits. One question in implementing HiSSE is how to set the transition parameters among states (combination of observed 0/1 and hidden A/B states, with combined state space 0A, 1A, 0B and 1B). We followed suggestions of the authors of the method (Beaulieu & O'Meara, 2016), either setting all transition rates to be equal, or assumed a three-parameter rate in which transitions between the observed states could vary but transitions between hidden states is a single parameter. The CID-2 and CID-4 models are fit including two or four hidden states, respectively, but with no dependence on the observed traits. For these, we also assumed alternatively a single rate for all state transitions (observed and hidden) or a three-rate model including two rates for transitions between the observed states and one between all hidden states.

We implemented all of the above analyses using functions in the R package *hisse* (Beaulieu & O'Meara, 2016). As with the BAMM analysis, we ran all models using each assumption about sampling completeness (L, M, or H), and analysed both the global *Pheidole* and New World only. For the New World analyses, we additionally adjusted the sampling fraction (M*) to account for possible undersampling of tropical species relative to extratropical species. As the ML optimization does not always find the global minimum from a single starting point, we ran 20 ML searches for each model using random starting parameters chosen from a uniform distribution on the interval (0,1). For all the models above, we ran them alternatively assuming a fixed root in the tropical state, or root probability estimated with the default "madwitz" method based on the data. As we found the results were insensitive to the root method, we only present results with the default option. After all BiSSE, HiSSE and CID models were inferred, we compared all models with sample-size corrected Akaike's information criterion (AICc) scores.

3.2.3 | FiSSE approach

FiSSE (Rabosky & Goldberg, 2017) is a nonparametric test for trait-dependent speciation rates that does not assume an underlying model structure, but rather depends on distributions of branch lengths in the different states. FiSSE is complementary to the BiSSE and is robust to Type-I error. We performed both one-tailed and two-tailed tests of FiSSE to test for speciation differences between extratropical and tropical taxa, using the global *Pheidole* and only the New World *Pheidole*. We also performed FiSSE on a set of trees for the New World only where temperate species were thinned to account for possible undersampling of the tropics (see Appendix S1, Supporting Information).

3.2.4 | Phylogenetic niche conservatism

While previous studies have shown that *Pheidole* likely has a tropical ancestor (Economo, Klimov, et al., 2015; Moreau, 2008), it remains an open question whether phylogenetic niche conservatism is strong enough to produce a gradient pattern during the Oligocene period, a key predictions of the TCH. We performed analyses to evaluate the degree to which latitudinal affinity is phylogenetically conserved in *Pheidole*, and used simulations to test if that conservation is strong enough for a gradient to emerge given tropical ancestry alone. For this, we first calculated two measures of phylogenetic signal—Blomberg's *K* (Blomberg, Garland, & Ives, 2003) and Pagel's lambda (Pagel, 1999)—treating absolute latitudinal midpoint as a continuous trait, using the phylosig() function in the R package *phytools* (Revell, 2012). Second, to estimate the overall evolutionary rates, we fit models of discrete character evolution (treating latitudinal affinity as a binary variable) using the fitDiscrete() function in the R package *geiger* (Pennell et al., 2014). To visualize the evolution of latitudinal affinity, we performed 100 stochastic character maps on the empirical tree using the make.simmap() function, and plotted a summary of state probabilities with the function densityMap(), both from the *phytools* package. Finally, to estimate whether the inferred rate of evolution combined with tropical ancestral state is consistent with the observed richness difference even in the absence of diversity regulation and diversification rate differences, we simulated niche evolution on the empirical tree and maximum likelihood model with the sim.history() function from *phytools*. While tree shape and trait state are not necessarily independent (i.e. the dependent model is implemented in the BiSSE/HiSSE analyses), this analysis asks whether we would be likely to observe a gradient even if they were independent, given that *Pheidole* likely has a tropical ancestor and given the rate that latitudinal affinity evolves. *Pheidole* likely has a tropical ancestor as *Pheidole fimbriata*, which is sister to the rest of *Pheidole*, and the sister lineage of *Pheidole*, *Cephalotes* + *Procryptocerus*, are all tropical (Moreau, 2008; Ward et al., 2015).

4 | RESULTS

Pheidole exhibits an LDG that is overall similar to ants as a whole (Figure 1). The BEAST analysis inferred a phylogeny whose major features are consistent with previous studies (Figure 2, Supporting Information Figure S1). The crown age of the group [i.e. the most recent common ancestor (MRCA) of *Pheidole fimbriata* with the rest

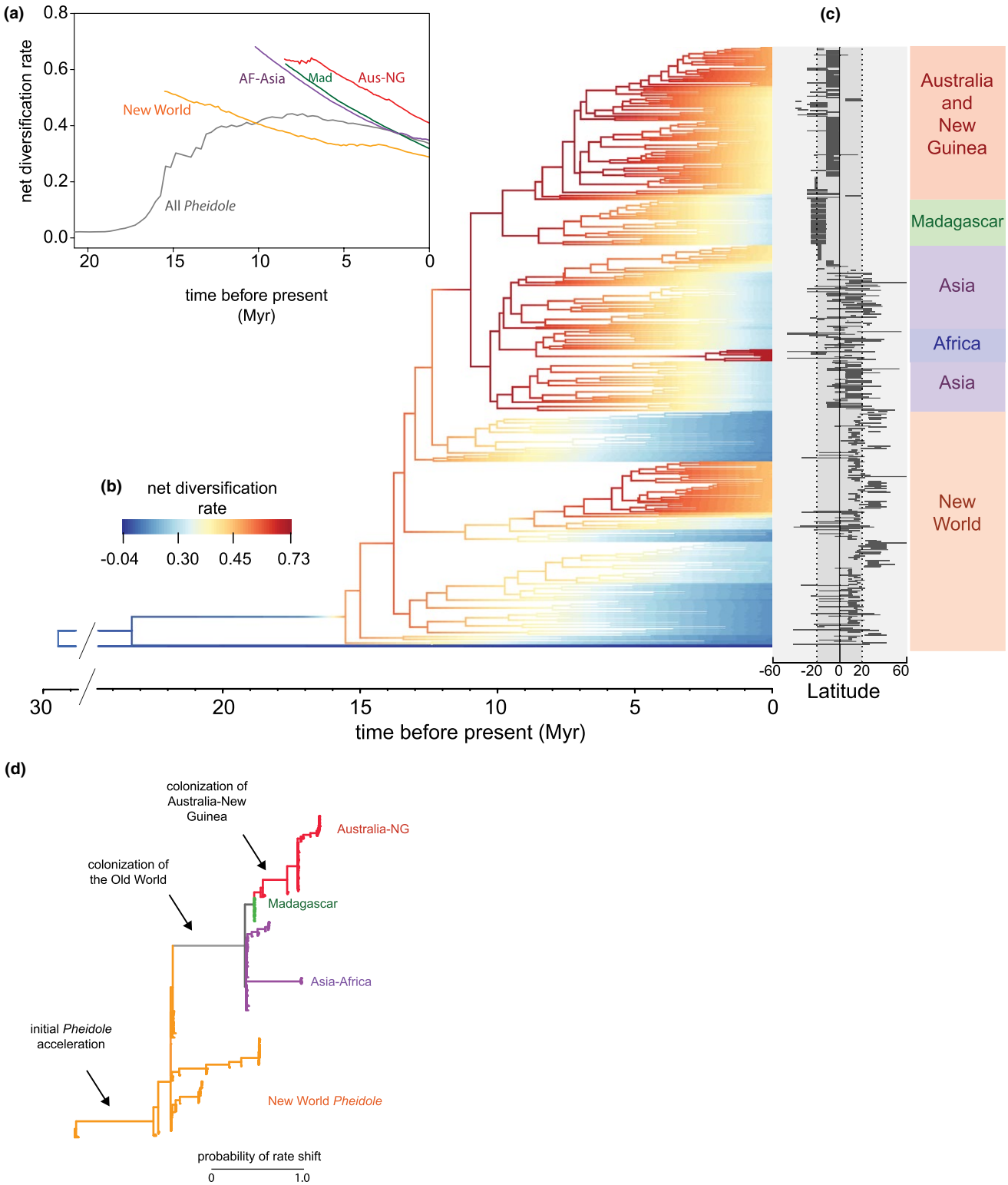

FIGURE 2 Diversification rate dynamics inferred with Bayesian analysis of macroevolutionary mixtures (BAMM) from a phylogeny of 449 *Pheidole* species. (a) Median diversification rates through time of the major *Pheidole* radiations, (New World, Africa-Asia, Madagascar, Australia-New Guinea). The New World median excludes the two early branching species (*P. rhea* and *P. fimbriata*) that fall outside the initial acceleration of *Pheidole* diversification. (b) The maximum clade credibility phylogeny coloured with inferred net diversification rate. (c) Latitudinal extent of all 449 taxa included in the tree. A high-resolution version with taxon names visible is presented in Supporting Information Figure S1. (d) Probable locations of diversification rate shifts, with branch length proportional to probability of a shift

of *Pheidole*] is inferred here to be younger than in a previous study (~29 vs. ~37 Ma in Economo, Klimov, et al., 2015).

According to the BAMM analysis, the hyperdiversification of *Pheidole* began after an acceleration approximately 15–16 Ma, and all extant species except for two early diverging lineages (*P. fimbriata* and *P. rhea*) are descended from this event (Figure 2). Diversification initially occurred in the New World, exhibiting a decelerating trend over time. Around 13 Ma, a single lineage colonized the Old World and this was associated with another burst of diversification followed by a slowdown in a clade encompassing Asia and Africa. Madagascar and Australia-New Guinea were later colonized, followed by accelerations and subsequent decelerations in each clade (Figure 2, Supporting Information Figures S1, S2). There were several other accelerations that were not obviously associated with geographic transitions, including one clade in the New World and the *megacephala* group in the Afrotropics. This general pattern of sequential colonization–acceleration–deceleration is robust to changing the sampling fraction parameter, although as one would expect, the inferred degree of deceleration becomes less pronounced if one assumes that more species are left to be sampled. However, there is no evidence that diversity is saturated (i.e. net diversification rate approaching zero) as net diversification rates were all strongly positive (ranging between 0.2–0.5 across regions and analyses with different parameter assumptions).

The extratropical lineages generally belong to young clades nested within larger tropical clades (Figure 2, Supporting Information Figure S1). While diversification rates vary across the genus to a degree, we could not detect a significant correlation (assessed with STRAPP) between BAMM-inferred net diversification rate and either absolute midpoint latitude or tropicality index across any of the analyses we performed (Figure 3). These results were similar across variation in the assumed global sampling fractions, whether

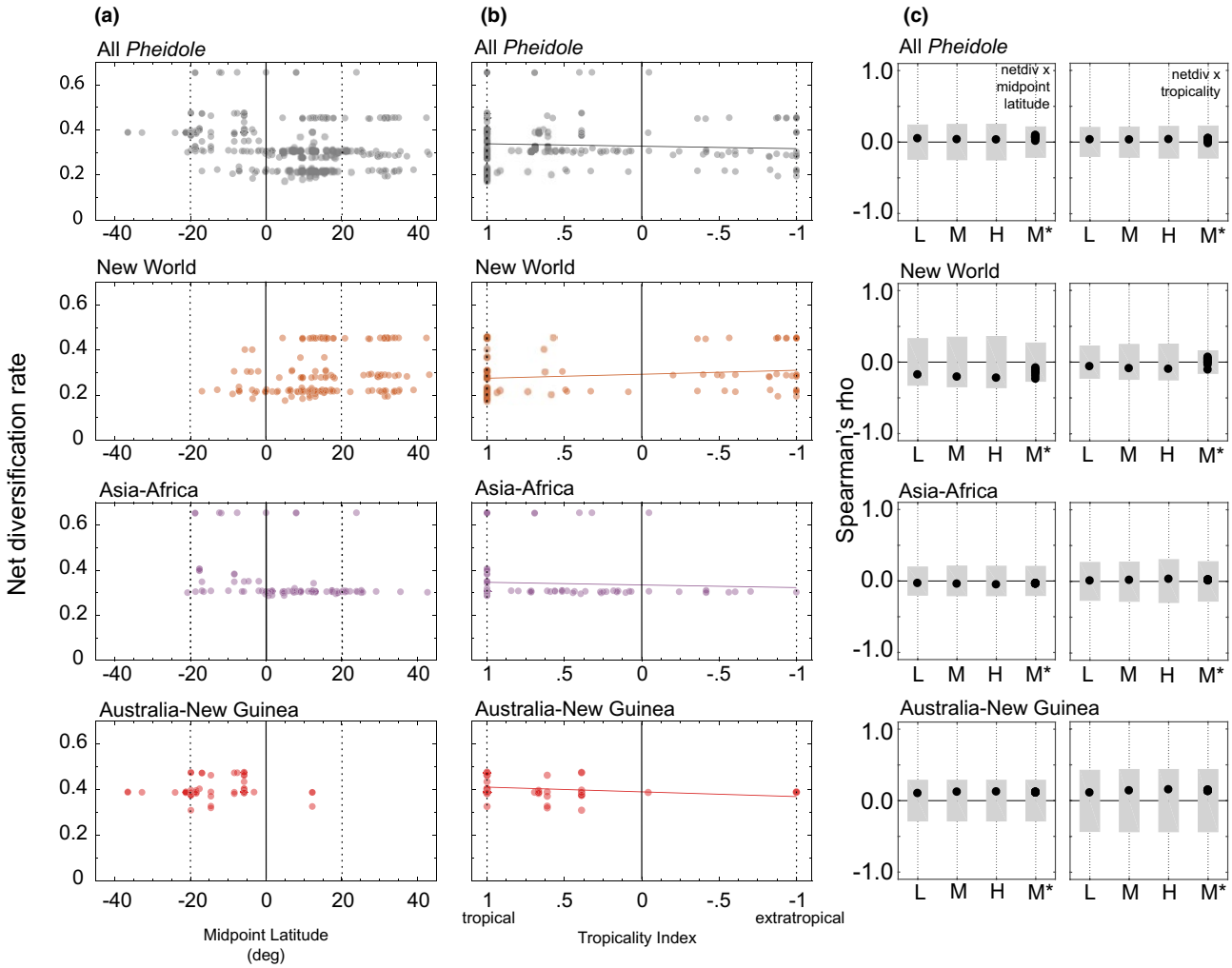

FIGURE 3 Net diversification rate inferred with Bayesian analysis of macroevolutionary mixtures (BAMM) as a function of latitude. Diversification rate of each *Pheidole* species (present day) inferred with BAMM using the "M" assumption of sampling completeness per species (a) as a function of latitudinal midpoint and (b) tropicality index, which varies from −1 for a species with a range located completely outside the tropics to 1 for a species confined to the tropics. (c) Spearman correlations (black dots) for net diversification and either absolute midpoint latitude (left) or tropicality (right), where the grey boxes reflect 95% null distribution generated with STRAPP. L, M and H reflect different assumptions about unsampled species (low, medium, high estimates of total numbers of *Pheidole*, respectively), while M* are 10 trees where temperate species have been culled to account for possible sampling bias (see Methods)

we calculated correlations for individual clades or the whole tree, and including trees where Nearctic species were culled to account for possible uneven sampling. Although significance tests were one-tailed for higher diversification in the tropics, we also note that none of the observed correlation coefficients were outside the null range in either direction.

The HiSSE analysis was also broadly consistent with the BAMM analysis in finding no statistical support for a correlation between macroevolutionary rates and latitude. In general, the CID-2 trait-independent null model outperformed the BiSSE trait-dependent models, and the CID-4 null outperformed the HiSSE trait-dependent models, and the CID-4 models had the global minimum AICc across the different permutations of the analysis (Table 1). Thus, this analysis provided no evidence for latitude-dependent macroevolution in this genus. It is worth noting as well that the AIC-minimizing versions of the BiSSE and HiSSE models, which again were themselves not preferred over the null models, generally did not support higher diversification rate in the tropics. The BiSSE model detected a slightly higher diversification rate in the extratropical zone and the HiSSE model either fit models where tropical diversification was higher than extratropical while in one hidden state and lower in the other hidden state, or where the extratropical diversification was always higher in both hidden states. For the New World, use of the sampling effort correction removed this slight, and non-significant difference. The GeoSSE analysis showed overall similar results to BiSSE, with a positive latitude–diversification rate trend in the New World, but not global, *Pheidole*; however, the association is not robust to the correction for latitudinal undersampling (see Supporting Information).

The FiSSE analysis was also consistent with the other analyses in showing no correlation between speciation rate and latitude for global *Pheidole* (λ_{temp} = 0.28, λ_{trop} = 0.27, two-tailed $p > .88$), but did show a positive speciation-latitude correlation for the New World alone (λ_{temp} = 0.30, λ_{trop} = 0.20, two-tailed $p < .026$). However, when we dropped extratropical tips from the phylogeny to simulate potential latitudinal undersampling of the tropics, this difference was much more modest and no longer significant (n = 10, mean λ_{temp} = 0.24, SE = 0.005, mean λ_{trop} = 0.20, SE = 0.0002, p range: .19–.72 among replicates).

The extratropical lineages are clustered with each other on the tree, although it is clear there were numerous transitions out of the tropics (Figure 4). The tests for phylogenetic signal in latitudinal affinity for Blomberg's K (K = 0.34, $p < .002$) and Pagel's lambda (λ = 0.95, $p < 10^{-57}$) were both highly significant. Symmetric and asymmetric models of discrete character evolution both fit the data comparably well (symmetric model $q_{trop\rightarrow etrop} = q_{etrop\rightarrow trop}$ = 0.015, AICc = 235.5, asymmetric model $q_{trop\rightarrow etrop}$ = 0.013, $q_{etrop\rightarrow trop}$ = 0.060, AICc = 234.9). Simulations of character evolution on the empirical phylogeny show that a latitudinal gradient is the most common outcome if one assumes a tropical ancestor and either model for the inferred rate of evolution of latitudinal affinity (Figure 4).

TABLE 1 Summary of sample-size corrected Akaike's information criterion (ΔAICc) from the binary state speciation and extinction (BiSSE) and hidden state speciation and extinction (HiSSE) trait-dependent models, and the two null models, character-independent models 2 and 4 (CID-2, CID-4)

		Global *Pheidole* (ΔAICc)			New World *Pheidole* (ΔAICc)			
Model	Description/constraint	L	M	H	L	M	H	M*
BiSSE	Div. rates and transition rates equal across latitudes	69.9	67.4	69.6	18.3	23.2	27.8	23.2
BiSSE	Div. rates equal, transition rates vary with latitude	69.4	66.9	69.2	19.4	24.2	28.9	22.3
BiSSE	Div. rates vary, transition rates equal with latitude	69.9	67.4	69.6	18.3	23.2	27.8	23.2
BiSSE	Div. rates and transition rates vary with latitude (full BiSSE model)	73.5	71.1	73.3	11.6	16.1	18.9	23.2
CID-2 null	Two hidden states, one transition parameter	33.9	21.9	17.3	0.5	2.8	5.7	2.9
CID-2 null	Two hidden states, three transition parameters	21.2	20.1	19.4	2.4	5.3	8.6	4.5
HiSSE	Div. rates vary with latitude and two hidden states, one transition parameter	36.1	21.1	16.5	0.9	0.9	0.3	8.6
HiSSE	Div. rates vary with latitude and two hidden states, three transition parameters	22.5	28.0	27.7	0.2	2.0	2.3	9.5
CID-4 null	Div. rates vary with four hidden states, one transition parameter	22.5	11.1	4.6	1.6	**0.0**	**0.0**	**0.0**
CID-4 null	Div. rates vary with four hidden states, three transition parameters	**0.0**	**0.0**	**0.0**	**0.0**	1.1	0.4	5.7

Note. Div. = diversification. CID-2 is similar in model complexity to the BiSSE model, while CID-4 is similar in model complexity to the HiSSE model. The models were run with different parameter constraints listed below. L, M and H refer to the low, medium and high estimates of missing taxa, respectively. M* includes a correction for possible oversampling with latitude. The AICc minimizing model for each analysis is highlighted in bold.

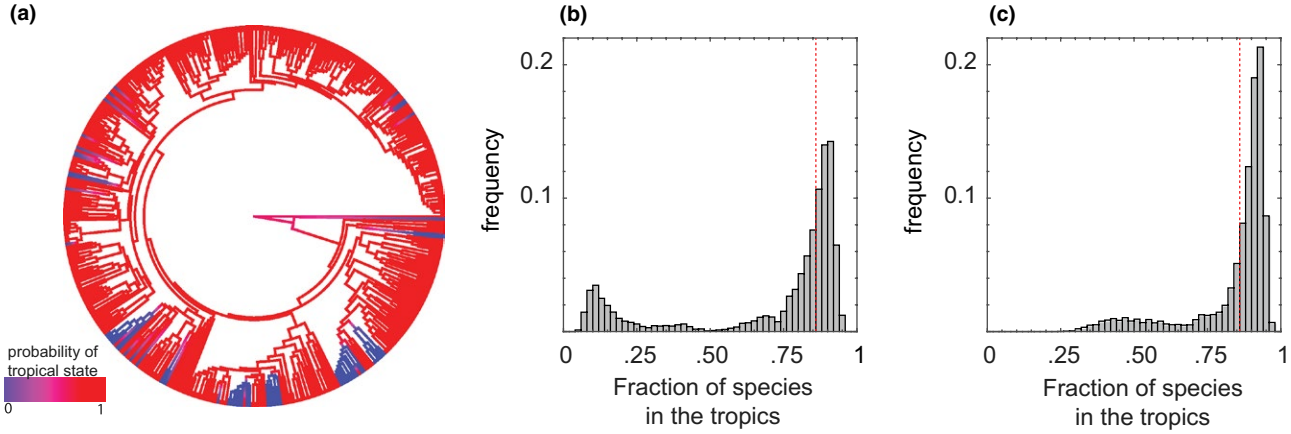

FIGURE 4 Evolution of latitudinal affinity in *Pheidole*. (a) Branch-wise probability of ancestral tropical state inferred from stochastic character mapping. (b)–(c) Histograms of latitudinal richness differences between tropics and extratropics simulated with stochastic character mapping on the empirical phylogeny assuming a tropical ancestor and the inferred degree of niche conservation using symmetric (b) or asymmetric (c) models of character evolution. The vertical dashed line is the empirical richness fraction

5 | DISCUSSION

Our analysis of *Pheidole* macroevolution sheds light on the mechanisms responsible for the evolution of the LDG in ants. By focusing on the dynamics of a massive radiation in the post-Oligocene, our study complements taxon-wide studies that focus on differences among highly divergent clades and deeper time-scales (e.g. Cardillo et al., 2005; Duchêne & Cardillo, 2015; Economo et al., 2018; Jetz et al., 2012; Kerkhoff et al., 2014; Pyron & Wiens, 2013; Rolland et al., 2014; Weir & Schluter, 2007).

We find no evidence of higher diversification rate for tropical *Pheidole* lineages across any of our analyses (Figures 2–4, Supporting Information Figure S1), as would be predicted by the DRH. In general, the signal of latitude as a trait affecting macroevolutionary rates in the BAMM, HiSSE, and FiSSE analyses was weak to non-existent. When there was some hint of a correlation, for example in the best-fitting (but still not better than null) HiSSE/BiSSE analyses, and the FiSSE analysis for New World speciation rate uncorrected for latitudinal sampling bias, it was in the direction of higher diversification/speciation in the temperate zone. However, those correlations were not robust to reasonable assumptions about undersampling in the tropics; thus, the overall picture is a lack of evidence for latitudinal dependency for macroevolutionary rates.

We do not view our analysis as ruling out that such systematic macroevolution–latitude relationships may exist, even in *Pheidole*. Rather, our analysis only suggests that such relationships are not the causal factor in the gradient. The DRH assumes that lineages reach different latitudes early on in their evolution, and the disparity of richness is due to different accumulation rates over time. If niche conservatism is too high for lineages to evolve out of the tropics (or vice versa) early on in the radiation, there may be no chance for any latitude–macroevolutionary rate correlations to manifest and be statistically detectible. Thus, we view our analysis as stronger evidence that a diversification rate–latitude correlation is not causal in the latitudinal gradient in *Pheidole*, rather than showing that no such relationship exists. The finding that a latitudinal gradient in diversification rates does not underlie the diversity gradient in *Pheidole* echoes similar results for birds (Jetz et al., 2012; Rabosky, Title, & Huang, 2015; Weir & Schluter, 2007), butterflies (Owens et al., 2017) and marine fishes (Rabosky et al., 2018; but see Siqueira et al., 2016). However, other recent work on mammals (Rolland et al., 2014), amphibians (Pyron & Wiens, 2013), and studies on the fossil record (e.g. Jablonski, Roy, & Valentine, 2006) indicated elevated net diversification rates in the tropics. Thus, there continues to be disagreement across studies and taxonomic groups. Whether this reflects true process variation across clades or differences in conceptual and methodological approaches across studies remains an open question.

Contemporary net diversification rates are positive in *Pheidole* across all regions, with current rates varying between 0.25 and 0.50 across regions and assumptions about missing taxa. This contradicts a key proposition of the ERH that diversity is at an equilibrium "carrying capacity" across regions (Hurlbert & Stegen, 2014b; Pianka, 1966; Rabosky & Hurlbert, 2015). There is evidence, however, that ecological opportunity at least partially controls diversification rate in *Pheidole*. Specifically, each time a new continent is colonized, diversification initially increases followed by a slowdown as richness increases, which can be a sign of niche filling (but not necessarily, see discussions in Moen & Morlon, 2014; Harmon & Harrison, 2015). This could indicate that diversity will eventually saturate at a steady state if net diversification rate continues to decrease (at current rates of rate decrease, this would occur in about 10–20 million years). However, diversity dependence is not in itself evidence of ecological limits (Harmon & Harrison, 2015), and it is equally plausible that richness would not saturate but instead reach a peak and then decline, resulting in a pulse or boom-bust pattern for the clade (e.g. as envisaged by Ricklefs, 2014). The further investigation of the role of ecological limitations on diversity, and any latitudinal differences

in those limits, remains an important direction for future work. One promising direction from a completely different angle would be to examine how niche overlap and coexistence in *Pheidole* varies with latitude or energetic constraints, as has been pursued in better studied taxa, such as birds (e.g. Pigot et al., 2016), but the ant data are not yet available at high enough resolution to pursue such analyses.

Overall, the results match the predictions of the TCH. We found that latitudinal affinity is moderately conserved in *Pheidole*. While there has been a number of transitions from the tropics to the temperate zone, latitudinal affinity evolves slowly enough to make a richness gradient the most likely outcome simply due to tropical ancestry and phylogenetic inertia. Thus, our study joins a series of recent studies supporting the TCH for woody plants (Kerkhoff et al., 2014), birds (Duchêne & Cardillo, 2015), mammals (Buckley et al., 2010) and butterflies (Hawkins & DeVries, 2009).

These results for *Pheidole* evolution in the post-Oligocene connect well to results on ant diversification on deeper time-scales (Economo et al., 2018), and together tell a coherent story about the evolution of latitudinal gradients in ants across scales. Most ant lineages older than 34 Ma are reconstructed to be tropical, including the *Pheidole* stem lineage. Around 15 Ma, *Pheidole* exhibited a manyfold acceleration in diversification rate and began a massive radiation. The reason for this initial acceleration, such as evolution of a key innovation, remains unknown. It took time for some *Pheidole* lineages to evolve the requisite traits for colonization of high latitudes. Once colonization of cold biomes occurred, diversification was not detectably slower. In their analysis across all ant clades, Economo et al. (2018) also found no evidence for elevated net diversification rates among clades centred in the tropics relative to those in the temperate zone, although clades are quite heterogeneous in rate, probably due to other latent biological and historical differences. It remained possible that diversification rate was correlated with latitude within the large clades, but biological differences among clades obscured this pattern. Within *Pheidole*, diversification rate is much less heterogeneous, but there is still no evidence of a negative latitudinal correlation, implying that lack of phylogenetic resolution within large clades was not hiding this relationship in the previous analysis (Economo et al., 2018). Further work is needed to unravel diversification patterns of other hyperdiverse ant clades (e.g. *Camponotus*, *Strumigenys*, *Tetramorium*, *Crematogaster*) that also exhibit strong latitudinal gradients to confirm this apparent consistency across phylogenetic scales. Indeed, these five hyperdiverse genera (out of 334 total ant genera) constitute over a third of all described ant species (~5,000), so each has a marked effect on ant-wide patterns such as latitudinal gradients.

While our results are most consistent with tropical niche conservatism (TCH) explaining the *Pheidole* latitudinal gradient, other patterns, such as the diversification pulses after colonization of new regions cannot be explained by phylogenetic niche conservatism alone. Rather, even though our study rejects the main assertion of the ERH that contemporary richness is regulated in steady state—*Pheidole* richness is apparently far from equilibrium everywhere—we find evidence for an underlying mechanism of the ERH that diversity and diversification are regulated by ecological opportunity and the filling of niche space. This underscores the point that the mechanisms underlying the broad umbrella hypotheses are not mutually exclusive, and even though a particular mechanism may not be causal for the gradient, it may still be an important process operating in the diversification of different groups, or operate on different phylogenetic scales in the same group (Graham et al., 2018). Further quantitative approaches may be necessary to disentangle different mechanisms operating simultaneously. We agree with the approach advocated by some (Hurlbert & Stegen, 2014a) towards a quantitative formulation of multiple competing and intersecting hypotheses, combined with a simulation-based approach to identify their key predictions. We felt initial efforts in this direction were not yet mature enough to use as a basis for the current study, but look forward to further development of the approach in the future.

Despite the high level of research effort directed toward understanding the latitudinal gradient, the matter is far from resolved (Mittelbach et al., 2007). Studies have differed in their conclusions about the origins of the gradient, probably due to both differences in conceptual and methodological approaches and real variation in process and history across taxonomic groups. The former should continue to improve as we develop more penetrating quantitative methods that make use of more diverse data types. Variability across taxonomic groups is best assessed and understood by examining more of them. With development of global invertebrate datasets like the one analysed here, we stand to broaden our perspective on large-scale biological patterns and their origins.

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DATA ACCESSIBILITY

Molecular sequences have been deposited in GenBank (see Supporting Information Table S1 for accession numbers). We have also provided the alignment, BEAST xml file and geographic dataset in a supplemental data archive (Dryad 10.5061/dryad.859nv4m). The GABI dataset can be accessed on the interactive website antmaps.org.

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BIOSKETCH

The research team is interested in the ecology and evolution of biodiversity, especially insects.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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