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Tese de Doutorado

Evolução temporal e espacial de formigas Ponerinae

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Evolução temporal e espacial de formigas Ponerinae

Maico Stochero Fiedler

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“Looking to geographical distribution, if we admit that there has been during the long course of ages much migration from one part of the world to another, owing to former climatal and geographical changes and to the many occasional and unknown means of dispersal, then we can understand, on the theory of descent with modification, most of the great leading facts in Distribution.”

Charles Darwin – The Origin of Species (1859)

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SUMÁRIO

RESUMO

Estudos ecológicos e evolutivos que incorporam grandes escalas de tempo e espaço nos permitem compreender como eventos passados, como tectonismo e extinções massa, por exemplo, moldaram a atual distribuição e abundância das espécies. Para além da evolução no tempo e espaço, outro fator determinante para a evolução das linhagens é a presença de atributos que possibilitem a sobrevivência e reprodução das populações ao longo do tempo. Utilizando um grupo de formigas para estudar os tópicos mencionados acima, a presente tese se divide em três partes. No capítulo I, de caráter mais exploratório, buscamos elucidar como se deu a evolução temporal de Ponerinae, com enfoque em como unidades geográficas distintas (biorregiões) afetaram a evolução das formigas. Também, buscamos entender como a diversificação pode afetar a diversidade de formigas ao longo de diferentes escalas espaciais analisadas. Já no capítulo II, testamos se a evolução de Ponerinae foi influenciada por dinâmicas ecossistêmicas ocorridas após o evento de extinção do Cretáceo-Paleógeno, como previamente sugerido na literatura cientifica. Contrário a essa ideia previamente sugerida, demonstramos que a evolução de Ponerinae se deu através de um contínuo aumento das taxas de diversificação ao longo do tempo. Por fim, no capítulo III, demonstramos que, apesar de ser relativamente rara em formigas, a reprodução das colônias por operárias (chamadas gamergates) ao invés de pela rainha não leva às espécies a um beco sem saída evolutivo. Ou seja, não encontramos evidência que espécies que apresentam reprodução por operárias tenham mais chances de serem extintas que espécies com reprodução pela rainha.

Palvras-chave: Macroevolução, taxa de diversificação, biorregião, gamergate, diversidade

ABSTRACT

Ecological and evolutionary studies that incorporate large scales of time and space allow us to understand how past events, such as tectonism and mass extinctions, for example, have shaped the current distribution and abundance of species. Beyond evolution over time and space, another crucial factor for lineage evolution is the presence of attributes that enable the survival and reproduction of populations over time. Using a group of ants to study the aforementioned topics, this thesis is divided into three parts. In Chapter I, of a more exploratory nature, we sought to elucidate the temporal evolution of Ponerinae, with a focus on how distinct geographical units (bioregions) influenced the ants' evolution. Additionally, we aimed to understand how diversification can impact ant diversity across different spatial scales analyzed. Moving on to Chapter II, we tested whether the evolution of Ponerinae was influenced by ecosystem dynamics that occurred after the Cretaceous-Paleogene extinction event, as previously suggested in the scientific literature. Contrary to this previously proposed idea, we demonstrated that the evolution of Ponerinae occurred through a continuous increase in diversification rates over time. Lastly, in Chapter III, we demonstrated that, despite being relatively rare in ants, colony reproduction by workers (called gamergates) instead of the queen does not lead species to an evolutionary dead-end. In other words, we found no evidence that species exhibiting worker reproduction have a higher risk of extinction compared to species with queen reproduction.

Key-words: Macroevolution, diversification rate, bioregion, gamergate, diversidade

LISTA DE TABELAS

Capitulo 1

Table S1. Genus richness and distribution across eleven bioregion used in this study. Occurrence data from Guénard et al. (2017) and genus richness from Bolton (2023). Af: Afrotropical, Au: Australian, Ne: Neotropical, Na: Nearctic, SJ: Sino-Japanese, Oc: Oceanian, Or: Oriental, Ma: Madagascan, Pa: Palearctic, Fi; Fiji and Ga: Galapagos.

Capitulo 3

Table 1. Summary of best-fitted hisse models. λ= speciation rate, μ= extinction rate, q= transition rate. *0A* represents a state where the gamergate trait is absent. *1A*, represent state where gamergaete is present disregarding a hidden state, and in *1B* represents presents a gamergate state considering a hidden state.

Table S1. List of 65 Ponerinae species with gamergate workers.

Table S2. Akaike model selection of 13 HiSSE models of stem trees. Models one to three does not present a hidden state. τ (tau) = speciation + extinction rate, ε = extinction fraction (extinction/speciation) and, q = transition rate. 0 and 1 after model parameters represent absence or presence of the gamergate trait, respectively. A and B after model parameters mean a not hidden or hidden state, respectively. Models that consider an irreversible transition from gamergate to non-gamergate (q1A→0A irr.) present the other transition as free. Models 1-3 does not present a hidden state (1B). AIC and weight values are represent by the mean and standard deviation of 100 phylogenetic trees. *P* refers to the number of parameters in each model.

Table S3. Akaike model selection of 13 HiSSE models of crown trees. Models one to three does not present a hidden state. τ (tau) = speciation + extinction rate, ε = extinction fraction (extinction/speciation) and, q = transition rate. 0 and 1 after model parameters represent absence or presence of the gamergate trait, respectively. A and B after model parameters mean a not hidden or hidden state, respectively. Models that consider an irreversible transition from gamergate to non-gamergate (q1A→0A irr.) present the other transition as free. Models 1-3 does not present a hidden state (1B). AIC and weight values are represent by the mean and standard deviation of 100 phylogenetic trees. *P* refers to the number of parameters in each model.

LISTA DE FIGURAS

Capitulo 1

Figure 1. Ancestral range estimation for Ponerinae plotted on a consensus tree. Letters in the center of the nodes shows the most probable range during each cladogenesis, with the oldest nodes denoting an Indomalayan or American origin of Ponerinae. Arrows represent some of the major dispersal routes indicated on the tree. Gray rectangles show major internal Ponerinae groups, from Schmidt (2013) and species richness from Bolton (2023). Branches in gray represent the outgroups, used to best calibrate the reconstruction modeling. Paleogeographic maps from Blakey (2008).

Figure 2. Spatial distribution of Ponerinae diversity. (a) species richness, (b) harmonic mean of diversification rate, based on 200 trees. Grid cell 2º x 2º. (c) latitudinal species gradient of Ponerinae (black line), with relatively homogeneous mean diversification rate along latitude (red line). (d) denotes the relationship between diversification and species richness by spatial cell (gray dots).

Figure 3. Ponerinae ant diversity and bioregions. (a) Bioregion delimitation based on Infomap analysis (species richness of each bioregion between parentheses). Bioregion nomenclature based on Holt et al. (2013). (b) variation in diversification rates across the eleven suggested bioregions, with each dot presenting the mean rate for each tree (200 trees). (c) archipelagian bioregions, on average, had higher diversification rates than continental or mainly continental bioregions . Regional richness is unrelated to diversification rate (d) but presents a non-linear relation with bioregion area (species-area relationship) (e).

Figure S1. Distribution of 30,286 coordinates points for 1,065 Ponerinae species. Data from the Global Ant Biodiversity Informatics - GABI (Guénard et al. 2017).

Capitulo 2

Figure 1. Diversification rate through time to the Ponerinae subfamily. In (a), the diversification rate in one sampled tree, just for visualization purposes. In (b) diversification through time for 15 stem trees, and in (c) for 15 crown trees. Vertical dashed line denotes K-Pg boundary about 66 million years.

Figure 2. Diversification scenario to internal major clades of Ponerinae based on 15 stem trees (b-h) and 15 crown trees (i-o). The phylogeny in (a) shows colors for each of the seven internal clades analyzed (b-h). The phylogenetic uncertainty was higher in some clades than others (e.g. *Leptogenys* and *Hypoponera*- f,h,m). The recent increase of diversification of Ponerinae seems to have been driven mainly by *Odontomachus* and *Ponera* groups (g,k,n). The dots in the inset chart show the mean diversification rate in each tree and net is the average to 15 trees. Vertical dashed line denotes K-Pg boundary about 66 million years.

Figure 3. Diversification rate through time to the Myrmicinae subfamily. In (a), the diversification rate in one sampled tree, just for visualization purposes. In (b) diversification through time for 10 stem trees and, in (c) for 10 crown trees.

Figure 4. Diversification trough time from CoMET analysis. Ponerinae diversification based on 15 stem (a) and 15 crown (b) trees. Vertical dashed line denotes K-Pg boundary about 66 million years. Bellow, the diversification to Myrmicinae, based on 15 stem trees (c) and 15 crown trees (d).

Figure S1. In (a), genus-level phylogeny of Ponerinae, indicating, by colors, the genus or genera that compose each internal major clade (Figure 2, in main text). Due to polyphyly of sister genera, *Anochetus* and *Odontomachus*, the *Odontomachus* lineage here, represent both genera. Green= *Odontomachus* genus group, brown= *Leptogenys*, orange= *Plectroctena* genus group, gray= *Hypoponera*, purple= *Ponera* genus group, blue= *Pachycondyla* genus group, black= *Plathytyrea*. To b-g, represent the simplified draft of uncertainty in relationship between OG and *Leptogenys* clade with external groups, black branches, not considered in diversification scenario of OG neither *Leptogenys* clade. Phylogeny from Economo et al. (2018).

Figure S2. Diversification through time to internal Ponerinae clades based on CoMET analysis. In (a) a sampled tree with branches painted illustrating each of seven internal clades (b-h). Each line in (b-h) represent one phylogenetic tree. Darker lines show stem trees and lighter lines, crown trees. Dashed vertical line denotes the Cretaceous-Paleogene extinction event.

Figure S3. Diversification rate to each of 15 crown trees of Ponerinae. To trees that present very high rate (>0.5), the clade with highest rate are highlighted by arrow, *Myopias* (black), *Euponera* (purple), *Mesoponera* (green), *Paltothyreus* (gray), *Diacamma* (darkblue), *Bothroponera* (yellow). Pl= *Plathytyrea*, PaG= *Pachycondyla* genus group, PoG= *Ponera* genus group, PG= *Plectroctena* genus group, Hy= *Hypoponera*, OG= *Odontomachus* genus group, Le=*Leptogenys*. Color bar indicate the net diversification rate.

Figure S4. Diversification rate to each of 15 stem trees of Ponerinae. To trees that present very high rate (>0.5), the clade with highest rate are highlighted by arrow; *Myopias* (black), *Euponera* (blue), *Mesoponera* (purple), *Pseudoneoponera* (gray), and, *Loboponera* (orange). Pl= *Plathytyrea*, PaG= *Pachycondyla* genus group, PoG= *Ponera* genus group, PG= *Plectroctena* genus group, Hy= *Hypoponera*, OG= *Odontomachus* genus group, Le=*Leptogenys*. Color bar indicate the net diversification rate.

Capitulo 3

Figure 1. One sampled tree, from Economo et al (2018), shows the distribution of gamergate (red branches) and non-gamergate lineages (blue branches) along Ponerinae tree. Although few species present the trait, gamergate evolved independently several times along Ponerinae evolution.

Figure 2. Distribution of model AIC and model weight to all models used here. In general, models that not consider a hidden state and models that consider a hidden state with irreversible transition from gamergate to non-gamergate presented bad fit. Model weight (a) and AIC values (b) to crown trees and model weight (c) and AIC values (d) to stem trees.

Figure S1. Net diversification rate based on models 4-7, to stem trees. State 1 represent species with gamergate trait, disregarding (1A) or not (1B) a hidden state. Each dot represent the mean diversification rate to each tree.

Figure S2. Net diversification rate based on models 4-7, to crown trees. State 1 represent species with gamergate trait, disregarding (1A) or not (1B) a hidden state. Each dot represent the mean diversification rate to each tree.

INTRODUÇÃO GERAL

Apresentação

Ao longo do meu doutorado investiguei a evolução de formigas Ponerinae, com enfoque em como o tempo, o espaço e atributos morfológicos influenciaram a diversificação dessas formigas. A tese é dividida em três partes. A primeira parte tem um sentido mais exploratório, sem teste direto de hipóteses, sobre como se deu a evolução da distribuição de Ponerinae e como o espaço afetou diversidade e diversificação dessas formigas. A segunda parte trata da evolução temporal de Ponerinae. Aqui, testei se a evolução de Ponerinae, conjuntamente com outro grande clado, Myrmicinae, ocorreu em sucessivas radiações, como previamente hipotetizado, ou se a diversificação segue um outro padrão distinto. Na terceira parte busco compreender melhor se atributos morfológicos das espécies podem aumentar a probabilidade de extinção de tais espécies, levando- as a um beco sem saída evolutivo (i.e. quando a emergência de um atributo gera uma taxa de extinção maior que a de especiação ao mesmo tempo que, uma vez presente, as linhagens não perdem esse atributo). Utilizo nesse caso, a evolução da reprodução por operárias, em espécies Ponerinae, para averiguar se tal distinto modo reprodutivo em organismos eussociais leva tais espécies para um beco sem saída evolutivo.

A distribuição das espécies no tempo

O número de espécies em um determinado clado varia de acordo com dois parâmetros; a taxa de formação de novas espécies (i.e. taxa de especiação) e a taxa de perda de espécies (i.e. taxa de extinção), que subtraídas, formam a taxa líquida de diversificação (especiação – extinção= diversificação). A taxa de diversificação pode ser positiva, se a taxa de especiação for maior que a taxa de extinção (número de espécies aumenta), ou ainda, pode ser negativa, se a taxa de extinção for maior que a taxa de especiação (número de espécies diminui).

Se as taxas de especiação e extinção se mantiverem constantes ao longo do tempo, o número de espécies do clado é dado pelo fator temporal, ou seja, se a taxa de especiação é maior que a taxa de extinção, o grupo vai acumulando espécies ao longo do tempo. Por outro lado, se a taxa de extinção for maior que a de especiação, o grupo vai perdendo espécies ao longo do tempo (Rabosky et al. 2012). Contudo, se a extinção e/ou especiação variam ao longo do tempo, o número de espécies do clado pode ser gerado por diferentes dinâmicas macroevolutivas. Por exemplo, se a taxa de extinção for mais alta que a taxa de especiação em por um determinado período de tempo, o clado sofrerá grandes reduções no número de linhagens, como aconteceu com os felídeos nos últimos 3 milhões de anos (Piras et al. 2018). Outra dinâmica possível é o aumento gradual da diversificação ao longo do tempo, gerando um aumento gradual da riqueza de espécies em direção ao presente, como encontrado em Cyatheaceae (Loiseau et al. 2020). Há, ainda, casos em que a diversificação aumenta rapidamente em um curto intervalo de tempo, gerando radiações evolutivas, onde várias novas espécies são formadas em um curto intervalo de tempo, como ocorreu com os canídeos sul-americanos (Porto et al. 2023).

O aumento da diversificação em radiações evolutivas pode ser causado tanto pelo aumento da taxa de especiação quanto pela diminuição da taxa de extinção, ou ambas (para uma discussão mais detalhada veja Rabosky & Lovette [2008]). Simplificadamente, o aumento da taxa de diversificação pode se dar pela maior oportunidade de isolamento e evolução alopátrica das populações (Simões et al. 2016). Isso inclui superar barreiras geográficas, como no caso dos tentilhões de Darwin (Losos & Ricklefs 2009), que podem ser acompanhadas de barreiras climáticas, como no caso de *Lupinus*, nos Andes (Hughes & Eastwood 2006). Ou ainda, pela evolução concomitante de novas linhagens e com diferentes características funcionais, levando as espécies a ocupar diferentes nichos, mesmo em simpatria (Rundell & Price 2009), como as aves da família Vangidae em Madagascar (Jønsson et al. 2012).

As radiações evolutivas também podem ser interpretadas em um contexto de cascatas tróficas (Brodersen et al. 2018). Isso é, o aumento da diversificação de presas ou hospedeiros pode induzir um aumento da diversificação de predadores ou parasitos. Tais eventos são melhor conhecidos nas relações inseto-planta (Moreau et al. 2005; Lopez-Vaamonde et al. 2006), mas exemplos em relações inseto-parasitoide (Abrahamson & Blair 2008) também são discutidos. Para formigas, Moreau et al. (2005) argumentam que a diversificação das formigas foi potencializada pela rápida proliferação das Angiospermas, aproximadamente 100 milhões de anos atrás. Adicionalmente, Wilson & Hölldobler (2005) sugerem uma interligada dinâmica predador-presa durante a evolução de Ponerinae, objeto de estudo da presente tese.

A distribuição das espécies no espaço

A partir do seu centro de origem geográfico (área onde surgiu a linhagem), as linhagens geralmente ampliam sua distribuição, não necessariamente de maneira linear e/ou contínua. Com a recente abundância de filogenias para os mais diversos grupos da árvore da vida, tornou-se possível reconstruir as rotas dispersivas de diversos clados de interesse, como mamíferos (Springer et al. 2011), plantas (Kim et al. 2019), insetos (Economo et al. 2014), diatomáceas (Pinseel et al. 2020), fungos (Codjia et al. 2023), entre outros.

Tais informações são relevantes para entender as possíveis causas e consequências da dispersão ao longo do tempo, incluindo sua relevância para o atual padrão espacial de riqueza do grupo. Assim, pode-se estimar, por exemplo, se uma determinada área atua ou atuou como fonte de espécies para área adjacentes (McCullough et al. 2022), se as linhagens colonizadoras ao dispersar para uma nova área experimentam um aumento de diversificação (Porto et al. 2023), ou ainda se a frequência e ou a idade que ocorreu a colonização influenciaram a atual riqueza de espécies local (Cai et al. 2020; Liu et al. 2021).

Ao longo da evolução geográfica de um grupo, as espécies, em geral, acabam por apresentar padrões de agrupamento no espaço. Isto é, para um determinado grupo, algumas espécies podem estar mais relacionadas à área "A" enquanto outras espécies do mesmo grupo estão relacionadas à área "B". Podemos denominar tais áreas de biorregiões, evorregiões ou ainda regiões biogeográficas (Holt et al. 2013; Edler et al. 2017; Maestri and Duarte 2020). Tais dissimilaridades na composição de espécies ou linhagens refletem certo grau de isolamento que as regiões apresentam ou apresentaram entre si ao longo das eras geológicas (Riddle & Hafner 2010). O isolamento pode ser dado por fatores geológicos, como a tectônica de placas e o soerguimento de montanhas, por fatores climáticos como a temperatura, pela capacidade dispersiva das espécies ou ainda pela disponibilidade de nicho (Holt et al. 2013; Ficetola et al. 2017).

A separação da diversidade em áreas geográficas delimitadas nos permite analisar a relativa contribuição de cada área para a evolução de um determinado grupo. Por exemplo, Matos-Maraví et al. (2021) demonstraram que, para borboletas Brassolini, a Mesoamérica age como um berçário (alta diversificação) de novas espécies. Já regiões de Mata Atlântica agem como "museus" de biodiversidade, com a presença de linhagens que lentamente acumularam espécies ao longo do tempo (Matos-Maraví et al. 2021). Isso demonstra uma evolução bioma-específica para Brassolini e, a partir disso, pode-se explorar quais as possíveis causas dessa especificidade evolutiva.

Assim, compreender como ocorreu a dispersão dos grupos e como foi moldada sua atual distribuição, bem como compreender a importância relativa de diferentes biorregiões para a evolução das linhagens nos traz um panorama mais completo sobre como as espécies evoluem no espaço.

Atributos fenotípicos e sua influência na diversificação

São várias as causas que geram a diversificação das linhagens. As causas podem ser abióticas, como mudanças geológicas e climáticas durante o soerguimento dos Andes, por exemplo (Hughes & Eastwood 2006), ou ainda bióticas. Dentre as causas bióticas, a presença, manutenção ou surgimento de variação fenotípica pode desencadear um aumento na probabilidade de especiação das espécies. Por exemplo, Blanchard & Moreau (2016) estimaram que formigas que apresentam colônias maiores também apresentam maiores taxas de diversificação, em comparação com formigas com menores colônias. O efeito do tamanho da colônia possivelmente se traduz numa maior capacidade defensiva (menor mortalidade das colônias) e maior divisão de tarefas (maior sobrevivência das colônias) (Blanchard and Moreau 2016)

Por outro lado, a emergência de um atributo pode aumentar a chance de extinção das linhagens, reduzindo ou mesmo tornando negativa a taxa líquida de diversificação de tais linhagens. Alguns exemplos incluem autofertilização em plantas (Day et al. 2016), evolução da sociabilidade em aranhas (Agnarsson et al. 2006) e isolamento reprodutivo via seleção sexual em pequenas populações de tentilhões (Brumm et al. 2010).

Assim, a evolução de um atributo pode desencadear um beco sem saída evolutivo. O conceito de beco sem saída evolutivo é um pouco fluído, sendo as vezes considerado quando a diversificação é reduzida devido a presença do atributo em questão (Day et al. 2016), Pode ainda ser mais restritivo, considerado apenas quando as espécies com o atributo apresentam diversificação negativa e uma vez que as espécies apresentam esse atributo, ele não é mais perdido ao longo do tempo (Takebayashi& Morrell 2001). Nesse caso, o surgimento de um atributo é positivamente selecionado em curto intervalo de tempo (poucas gerações), mas negativamente selecionado ao longo do tempo geológico, devido ao aumento da chance de extinção dessas espécies. No exemplo da autofecundação (Day et al. 2016), tal característica pode facilitar a reprodução dos indivíduos a curto prazo (seleção positiva), mas a longo prazo diminui a variabilidade genética, aumentando o risco de extinção.

Evolução temporal e espacial das formigas

Sob a ótica da sistemática filogenética, as formigas (família Formicidae) formam um dos clados de “vespas” (Hymenopera) que apresentam comportamento eussocial (Peters et al. 2017). As formigas se tornaram, possivelmente, os artrópodes de maior abundância e biomassa no planeta, com indivíduos estimados em $2x10^{15}$ (Schultheiss et al. 2022) representando aproximadamente 45% da biomassa total dos artrópodes e 6.5 vezes a biomassa dos vertebrados selvagens (Eggleton 2020).

Até o momento foram catalogadas 14.120 espécies de formigas, pertencentes a 16 subfamílias (Bolton 2023). A idade do clado permanece em debate, com estimativas, para a idade coronal do grupo (idade do ancestral de todas as espécies atualmente vivas do clado), que variam dos 112 aos 169 milhões de anos (Moreau et al. 2005; Brady et al. 2006; Moreau and Bell 2013; Schmidt 2013; Borowiec et al. 2019). Os fósseis de formigas sabidamente mais antigos, entretanto, datam de aproximadamente 100 milhões de anos, encontrados onde atualmente são a França e Myanmar (LaPolla et al. 2013).

Espacialmente, as formigas apresentam nítido gradiente latitudinal de riqueza de espécies (Economo et al. 2018), com as maiores diversidades sendo catalogadas para o sudeste asiático, Austrália, Congo, floresta Amazônica e América central continental (Guénard et al. 2017). Contudo, tal padrão espacial se deve a ação temporal sobre as linhagens de formigas. Ou seja, a maior incidência de espécies nas regiões tropicais deve-se ao fatos das linhagens tropicais serem mais antigas que as extratropicais, proporcionando maior tempo para acumular linhagens nos trópicos (Economo et al. 2018).

As formigas Ponerinae

Ponerinae é a terceira maior subfamília de formigas, e a maior dentre as formigas Poneromorfas (Keller & Peeters 2021). Atualmente inclui 1,274 espécies descritas em 50 gêneros (Bolton 2023). Uma típica Ponerinae nidifica sob cavidades no solo, na serapilheira ou em madeira em decomposição, preda artrópodes, e origina uma nova colônia de modo independente, isso é, a rainha dispersa sozinha para um novo local (Schmidt & Shattuck 2014). O tamanho das colônias em geral é pequeno se comparado às demais formigas, atingindo, geralmente, de dezenas a poucos milhares de operárias (Schmidt & Shattuck 2014). A variação morfológica entre operárias e rainhas também é considerada pequena (Schmidt 2013), o que é incomum para a maioria das demais formigas.

A origem da subfamília no tempo e espaço ainda é discutida, com alguns estudos datando sua origem por volta de 110-120 milhões de anos atrás (Moreau et al. 2005; Economo et al. 2018; Romiguier et al. 2022), enquanto uma origem mais recente, 61-85 milhões de anos, também é sugerida (Schmidt 2013; Borowiec et al. 2019). Utilizando uma quantidade limitada de espécies, Moreau & Bell (2013) indicam que a subfamília teria surgido no continente Americano e posteriormente dispersado para amplas regiões do planeta.

Assim como Formicidae, Ponerinae apresenta uma distribuição majoritariamente pantropical (Guénard et al. 2017). Schmidt (2013) subdivide a subfamília em seis grandes clados: *Platythyrea* (40 espécies), com distribuição pantropical, grupo *Pachycondyla* (125 espécies), com distribuição americana, grupo *Plectroctena* (55 espécies), com distribuição primariamente africana, grupo *Odontomachus* (710 espécies), com distribuição pantropical e *Hypoponera* (154 espécies), com distribuição pantropical e, grupo *Ponera* (167 espécies), com distribuição primariamente eurasiana e australiana.

Contudo, a dispersão da subfamília desde o Cretáceo permanece desconhecida. Isso se deve, em parte, pela ausência de uma robusta filogenia no nível de espécie e a ampla distribuição de alguns gêneros como, por exemplo, *Ponera*, *Hypoponera* e *Odontomachus*, que ocupam três ou mais continentes (Schmidt and Shattuck 2014). A evolução espacial de Ponerinae é explorada no capítulo I. Assim, no capítulo I busco explorar os padrões de distribuição de Ponerinae no espaço, com enfoque na relação diversidade-diversificação, como as espécies estão agrupadas no espaço e quais foram as possíveis rotas de colonização de Ponerinae ao longo dos últimos 100 milhões de anos.

A evolução temporal das Ponerinae também ainda permanece pouco explorada. A hipótese mais empregada para explicar a evolução do grupo foi proposta por Wilson & Hölldobler (2005). Os autores argumentam que após a extinção do Cretáceo-Paleogeno, cerca de 66,04 milhões de anos atrás, as florestas tropicais expandiram suas distribuições, o que desencadeou um aumento na fauna de artrópodes nessas regiões. Como predadoras de artrópodes, as formigas Ponerinae teriam apresentado um aumento da diversificação, pela grande oferta de presas. Contudo, durante o Eoceno, sua diversificação teria sido reduzida, por competição derivada da rápida diversificação das formigas Myrmicinae, competidoras superioras em relação às Ponerinae.

Wilson & Hölldobler (2005) chamaram essa hipótese da sucessão dinástica, a evolução, primeiramente de formigas socialmente mais simples, sendo posteriormente superada pela evolução de formigas socialmente mais complexas. Embora seja comumente utilizada para explicar a evolução de Ponerinae, a dinâmica evolutiva Ponerinae-Myrmicinae sugerida pela hipótese da sucessão dinástica não foi de fato testada. Assim, o capítulo II da tese busca testar se a evolução das Ponerinae ocorreu conforme proposto pela hipótese da sucessão dinástica.

Formigas Ponerinae foram historicamente consideradas como primitivas devido a algumas características morfológicas, tais como: baixa fecundidade da rainha, forrageamento solitário em algumas espécies, e baixo grau de dimorfismo entre rainhas e operárias (Wilson & Hölldobler 2005). Essa última característica é elencada como fator decisivo para a evolução de um comportamento que surgiu diversas vezes ao longo da evolução de Ponerinae: a reprodução por operárias (Blatrix & Jaisson 2000; Monnin & Peeters 2008; Peeters & Fisher 2016). Estipula-se que a alta similaridade morfológica entre rainhas e operárias se manifesta também em uma espermateca viável e capaz de gerar ovos fertilizados (futuras fêmeas), nas operárias, assim como ocorre nas rainhas (Blatrix & Jaisson 2000). Dessa maneira, as formigas operárias conseguem acasalar e gerar ovos fertilizados. Tais operárias são chamadas de gamergate (do grego “operária casada”) (Peeters & Crewe 1984). Atualmente, cerca de 6% das espécies de Ponerinae apresentam reprodução por operárias.

Contudo esse intrigante comportamento das operárias possivelmente gera desvantagens eco-evolutivas para as espécies. Por exemplo, Monnin & Peeters (2008) descobriram que espécies que se reproduzem via gamergate, em geral apresentam menor tamanho de colônia que espécies que se reproduzem unicamente via rainha. A variabilidade genética intraespecífica também é baixa entre algumas espécies estudadas com reprodução via gamergate (Viginier et al. 2004). E, dentro de uma mesma espécie, *Harpegnathos saltator*, Liebig & Poethke (2004) encontraram que colônias com reprodução via gamergate apresentam taxas de mortalidade quatro vezes maiores que colônias com reprodução via rainha.

Todos esses fatores sugerem que a reprodução via gamergate pode gerar consequências para a diversificação dessas espécies, reduzindo sua diversificação ou mesmo tornando a chance de extinção de tais espécies mais provável que a de especiação, ao longo do tempo. Tal cenário de diversificação negativa pode gerar um beco sem saída evolutivo, onde as espécies que apresentam a característica gamergate estão fadadas a se extinguirem ao longo do tempo. Assumindo esse cenário, no capítulo III da tese é investigado se gamergate leva as espécies Ponerinae para um beco sem saída evolutivo.

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PARTE I

A EVOLUÇÃO ESPACIAL DE PONERINAE

Capítulo 1

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Spatial evolution of Ponerinae: The role of geographic evolution on a large ant group

Spatial evolution of Ponerinae

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Abstract

Species diversity is unevenly distributed in geographic space. Geologic events (continental drift), species ecological features (dispersal capabilities), or macroevolutionary dynamics (diversification rates) are among the causes of such variation across space. Ponerinae is the third largest ant subfamily, encompassing about 1300 species with a primarily pantropical distribution. However, very little is known about its biogeographic evolution, patterns of diversity and diversification across geography. Here, we compiled the most comprehensive point-locality species distribution data for Ponerinae to date, explored its biogeographic evolution, and constructed bioregions to investigate how diversity, diversification, isolation, and area are correlated to each other across these bioregions. We found that in early Ponerinae evolution, the Indomalayan region acted as center of origin for the major internal Ponerinae clade, contradicting previous findings. Then, lineages spread to adjacent landmasses, mainly to the Afrotropics, following major dispersal routes along the pantropics and secondary dispersal routes through temperate zones. We found that Ponerinae species diversity is unrelated to diversification rates along latitudinal, bioregional, and assembly levels. Nevertheless, we found a faster diversification rate in archipelagic bioregions than continental ones, which may suggest that islands are important centers of origin for new Ponerinae ants. Continental landmasses present slower diversification rates, but harbor more species, following a non-linear species-area relationship.

Key words: ant, macroecology, biogeography, diversification

Introduction

Biological diversity exhibits a non-random distribution across space (Ficetola et al., 2017). A commonly studied driver of biodiversity patterns across space is the diversification rate (Cai et al., 2020; Liu et al., 2021). The diversification rate hypothesis assumes that the variation in species richness between two or more areas is directly triggered by the rate of diversification of lineages. That is, areas with higher diversification rates present high species richness (Condamine et al., 2012). Such pattern is observed, for example, in mountain versus non-mountain lineages (Camacho et al., 2021) and in the latitudinal diversity gradient (Mittelbach et al., 2007). The analysis of diversification in space also enables the recognition of possible “cradles of biodiversity”, regions with high emergence of new species, that act as a center of origin of species diversity (Moreau and Bell, 2013).

Before diversification, species distribution across space depends on which species or lineages first colonized the area. In this sense, information about dispersal routes throughout the evolution of a clade may clarify how lineages spread and diversified across space (Hughes and Eastwood, 2006). The reconstruction of ancestral ranges and the evolution of the biogeographic distribution of diversity enable us to infer which areas, in the past, probably acted as sources of lineages to other areas and the consequences of such dispersal to the evolution of clades (Roncal et al., 2013; Price et al., 2018). For example, Matos-Maraví et al., (2021) found that Brassolini, a Neotropical Lepidoptera clade, probably originated in the Amazon Rainforest and subsequently dispersed to other Neotropical regions. Moreover, given this dispersal dynamics, Brassolini slowly accumulated species in Atlantic Rainforest, while experienced an elevated diversification rate in Mesoamerica (Matos-Maraví et al., 2021). Thus, understanding temporal features of geographic evolution could improve the understanding of the current spatial distribution of biological diversity.

The distribution of diversity in bioregions or evoregions reflects the dissimilarity in diversity distribution, both species and phylogenetic dissimilarity (Edler et al., 2017; Maestri and Duarte, 2020). Such dissimilarity is often triggered by geological events (e.g.

plate tectonics), climate (e.g. variation in temperature along mountain ranges), dispersal capabilities, and niche suitability (Holt et al., 2013; Ficetola et al., 2017). From this, biogeographic regionalization enables us to identify if different areas present distinct evolutionary histories, based on past geographic and climatic evolution. For example, McCullough et al., (2022) found that corvids in the islands on the eastern side of Wallace's line present a higher diversification rate when compared to corvids from the western side; this reflects the role of geographic isolation between Australia and Asia to the evolution of such birds.

Ponerinae is the third-largest ant subfamily, reaching about 1.300 species (Bolton, 2023), with some studies estimating an age of about 110 to 120 million years (Moreau et al., 2005; Economo et al., 2018; Romiguier et al., 2022) or younger (~72 million years [Borowiec et al., 2019]). A typical Ponerinae species preys on arthropods, living predominantly on the ground or leaf litter and presenting small colony size, often from dozens to a few hundred workers (Schmidt and Shattuck, 2014). Moreau and Bell (2013) estimate a Neotropical origin to crown Ponerinae. From this, several dispersal events across continental and biogeographic regions appear to have occurred, leading to the occupancy of the pantropics, with temperate regions being less speciose (Schmidt, 2013). Besides, Ponerinae genera present high variation in spatial distribution, with some genera being more restricted (e.g. *Loboponera* and *Hagensia*, present only in portions of the Afrotropics) and some genera being globally spread, as *Leptogenys*, *Hypoponera*, and *Ponera* (Schmidt and Shattuck, 2014).

Being a highly speciose group with global distribution, Ponerinae is a good model to investigate patterns of diversity and diversification across space. As such studies generally use vertebrates (e.g. Jetz et al., 2012; Cai et al., 2020; Stevens et al., 2019), a study under an insect perspective may clarify similarities and dissimilarities of spatial evolution among terrestrial animals. Here, we seek to understand how diversity and diversification rates interact along the distributional range of Ponerinae. Breaking the distribution of Ponerinae into bioregions, we seek to estimate the relative importance of diversity in each region. We also seek to understand, in a wide overview, the possible effects of purported dispersal routes taken along the last 100 million years for the current distribution of these ants.

Material and Methods

Phylogenetic data

We used the comprehensive phylogenies of Formicidae from Economo et al., (2018), encompassing 14,512 species, of which 1,310 are Ponerinae species and subspecies, totaling about 91% of described species of this subfamily (Bolton, 2023). The phylogenies are built from two methods of insertion of missing species on backbone trees, built with molecular data. For the first method, missing species were randomly drafted, starting in the crown age of clades (usually genus) of a backbone tree. For the second, species insertion was made between the crown and stem age of the clade (See Economo et al., (2018) supplementary material, for more details). Following the authors' nomenclature, hereafter we refer to phylogenies as either crown (developed with the first method) or stem (second method) phylogenies.

Species distribution

Species distribution was obtained from the Global Ant Biodiversity Informatics-GABI database (Guénard et al., 2017). The GABI compiles recorded coordinates for ant species across the world with information, up to 2018, from 9.300 publications on about 15.000 ant species totaling 1.9 million records. For Ponerinae, the GABI presents about

66,000 occurrences encompassing all genera present in Economo et al., (2018) trees. To purify the data, we first removed dubious species identifications and oceanic coordinates. Second, we corrected coordinates with inverted signal value (e.g. species recorded as longitude 46º instead of the real location at -46º of longitude; we found dozens of such mistaken coordinates). Similar cases were also found confounding Australia with China (inverted latitudes). Third, we checked manually the coordinates of species present in two or more bioregions (see below in bioregion delimitation section). Such approaches also enabled us to remove remaining coordinates of exotic invasive species, since the dataset usually do not present coordinates for exotic distributions. Fourth, the dataset present several duplicate data (same species at same coordinates). We opt to remove duplicate records, keeping only unique coordinates, since we noted some resulting differences in bioregion delimitation (see below) when duplicate coordinates were considered.

About half of the occurrences compiled in GABI do not present a coordinate point, but a relatively detailed site of collection (e.g. municipality/county name), which includes about 33,000 records, including probably duplicate records. From those, we manually collected coordinate points, using the centroid of the smaller described local species collection site (e.g. if the information on species A is “collected in municipality x”, we use the centroid of such municipality as a geographical coordinate for such occurrence). Occurrences without more detailed information, such as “Amazon Rainforest” were not used. Thus, we increased distribution data for about 250 species, reaching 1,067 Ponerinae species and subspecies overall. Altogether, our sample size reaches 30,286 coordinate points (Figure S1).

Ancestral range estimation

We seek to estimate the ancestral range of the Ponerinae and the main routes of dispersion of major Ponerinae clades. Due to high phylogenetic uncertainty, mainly within genus, we opt to use a genus-level tree during estimation range. Although the author of the *BioGeoBEARS R* package (Matzke, 2013) does not recommend a genus-level phylogeny to estimate ancestral ranges, we argue that to trace the main dispersal events along Ponerinae evolution could be informative to explain the current wide distribution of this group. Thus, we used the Statistical Dispersal-Extinction-Cladogenesis (S-DEC) model (Ree and Smith, 2008) considering 200 alternative trees (compiling the 100 stem and 100 crown trees) to recreate the most probable biogeographic history of Ponerinae. The Statistical DEC enables to summarize the analysis for 200 trees in a consensus tree, estimating the more probable ancestral reconstruction scenario given detected phylogenetic uncertainty.

Due to phylogenetic uncertainly, we reduce the number of areas and follow a more traditional delimitation, encompassing only five biogeographic realms based on Cox, (2001): A: American, B: Australasian, C: Indomalayan, D: Afrotropical, E: Palearctic. We restrict dispersal routes between American and Palearctic realms as well as between Australian and the Palearctic. The biogeographical analysis was performed in RASP, version 4.2 (Yu et al., 2015).

Bioregion delimitation

To access the relative contribution of each bioregion to Ponerinae evolution, we defined the number of bioregions using the Infomap Bioregions approach (Edler et al., 2017). The Infomap algorithm aggregates species in grids generating a bipartite network with species and grids as nodes where each species is linked with the present grid cell (Edler et al., 2017) generating a link weight for each species on each grid cell. Each grid cell have 2º x 2º. Changing the cluster cost parameter in Infomap enables to alter the number of final bioregions; for our data, values smaller than 1.40 started creating several bioregions with only two or fewer species. Thus, we fixed the number of bioregions to 1.45, enabling bioregions with three or more species only.

Diversification rates across space

We use a tip rate measure of diversification rates to evaluate the evolution of Ponerinae in space. Tip rates commonly used include the inverse of equal split measure, the inverse of terminal branch length, and node density (Title and Rabosky, 2019). Due to phylogenetic uncertainty in branch lengths, we opted for a node density metric; since the first two measures consider branch length to calculate diversification rates, whilst node density considers the age of the tree and the number of nodes. Thus, the tip rate by node density is the number of splitting events along the path between root and tip of the tree divided by the age of the tree (Freckleton et al., 2008).

The diversification rate was calculated for each species in each phylogeny (100 crown and 100 stem) with 1310 species, and then, we extracted the rates for the 1067 species for which we have geographic distribution data. We estimated the diversification rate on the complete “1310 species trees” rather than “1067 species trees” to avoid bias on tree node numbers and, hence, diversification rates. The mean value of diversification for each species was very similar across stem and crown trees (correlation= 0,986), thus we compile the values of the 200 trees in a unique scenario rather than present the panorama of stem and crown trees separately.

Effects of Area

Finally, we investigate the species area relationship among bioregions. We extract the area of each bioregion using the function *st_area* from *sf* package (Pebesma, 2018). From this, we run a generalized linear model to investigate the species area relationship along Ponerinae bioregions.

Results

Historical biogeography

The DEC analysis estimates a probably American or Indomalayan origin for Ponerinae, since most of oldest tree nodes had an estimated origin in such bioregions (Figure 1). The Indomalayan was probably the area of origin of large Ponerinae clades (*Odontomachus*, *Plectroctena*, and *Ponera* groups) that posteriorly spread mainly to the Afrotropics and Australian regions, about 60-70 million years before present.

Posteriorly, the Afrotropics and American region probably became the center of origin of most current Ponerinae genera, given most of the living genera of the *Odontomachus* (~710 species) and *Plectroctena* (~55 species) groups seemed to originate in the Afrotropics while the *Pachycondyla* group (~125 species) likely evolved and spread along the Americans region.

Richness and diversification through space

Currently, the most speciose areas for Ponerinae are tropical regions, mainly in continental Central America, northern South America, Madagascar, tropical regions of Africa, and New Guinea islands (Figure 2a).

Mean diversification rate was relatively heterogeneous across space (ranging from 0.052 to 0.154 for each cell), with high rates estimated for Madagascar, the transition region between the Neotropic-Nearctic and portions of Australia. At the same time, most of the Afrotropical region and portions of Australia and New Zealand had smaller diversification rates when compared with other parts of the Ponerinae distribution (Figure 2b).

Ponerinae follows a latitudinal gradient in species richness that is uncorrelated with diversification rate (Figure 2c). The diversification rate is also unrelated to assembly diversity (grid cell) across space (Figure 2d).

Bioregions and regional richness

The distribution of Ponerinae was divided into eleven bioregions (Figure 3a), with the Oriental, Neotropical, and Afrotropical as the most speciose. Some islands and archipelagos were delimited as bioregions in themselves, including Madagascar and nearby islands, Fiji Islands, New Guinea and tropical Pacific islands (Oceanian), and the Galapagos archipelago.

Diversification across bioregions was relatively heterogeneous. On average, Neotropic, Afrotropic, and Australian regions presented the smallest diversification rates (Figure 3b). At the same time, the above island or archipelagoes bioregions (Malagasy, Fiji, Oceanian, and Galapagos) present a higher diversification rate when compared with continental bioregions (ANOVA, F=237.7, p<0.001) (Figure 3c). Despite variation in diversification rates across bioregions, such metrics do not explain the variation in species richness found along bioregions (Figure 3d), presenting a similar rate even among bioregions with wide variation in species richness.

Area

The species-area relationship presents a non-linear relationship, explaining about 60% of the variation in species richness found across eleven bioregions of Ponerinae distribution (p=0.002, R^2=0.595), with larger bioregions harboring more species than smaller bioregions (Figure 3e).

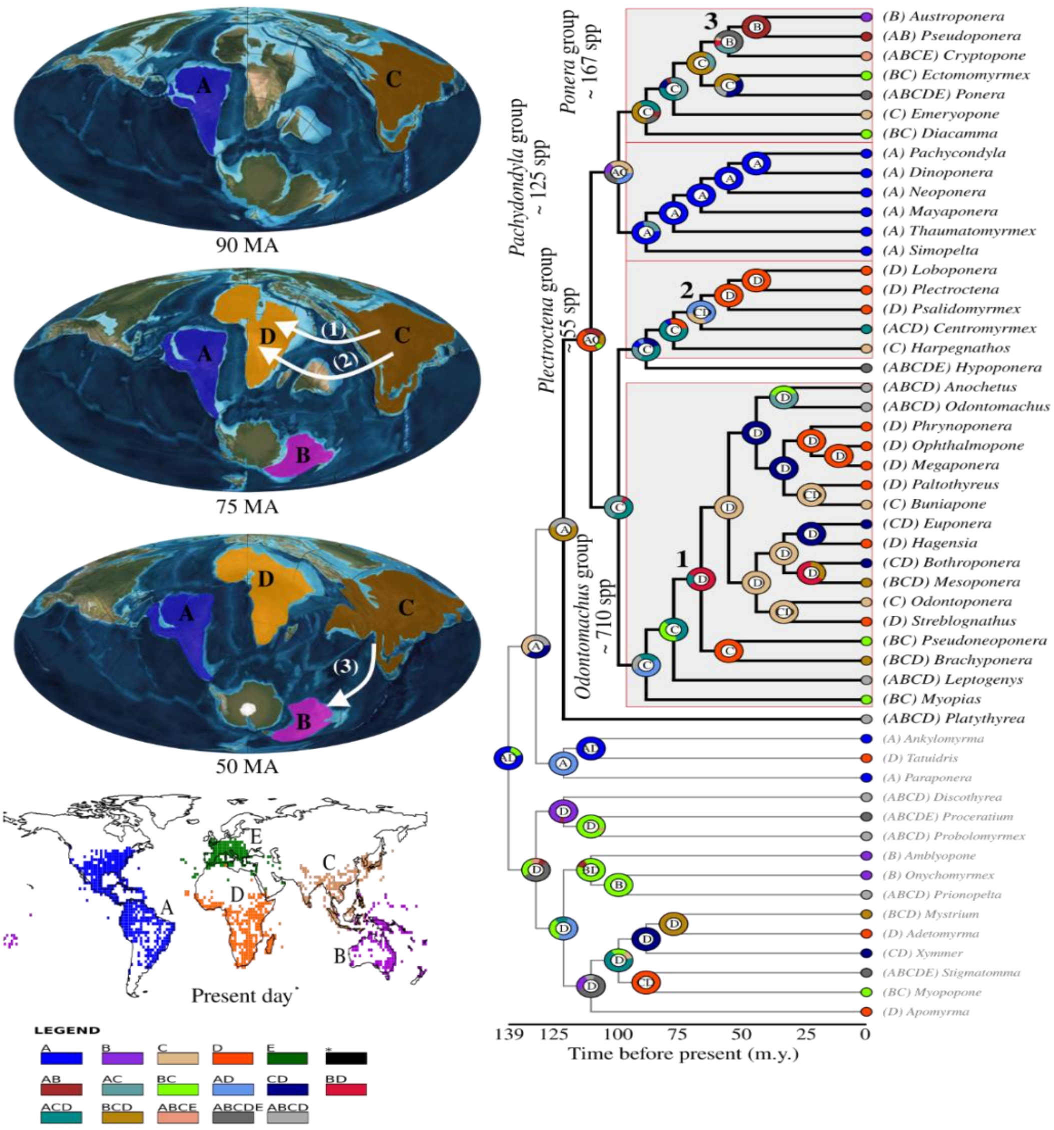

Figure 1. Ancestral range estimation for Ponerinae plotted on a consensus tree. Letters in the center of the nodes shows the most probable range during each cladogenesis, with the oldest nodes denoting an Indomalayan or American origin of Ponerinae. Arrows represent some of the major dispersal routes indicated on the tree. Gray rectangles show major internal Ponerinae groups, from Schmidt (2013) and species richness from Bolton (2023).

Branches in gray represent the outgroups, used to best calibrate the reconstruction modeling. Paleogeographic maps from Blakey (2008).

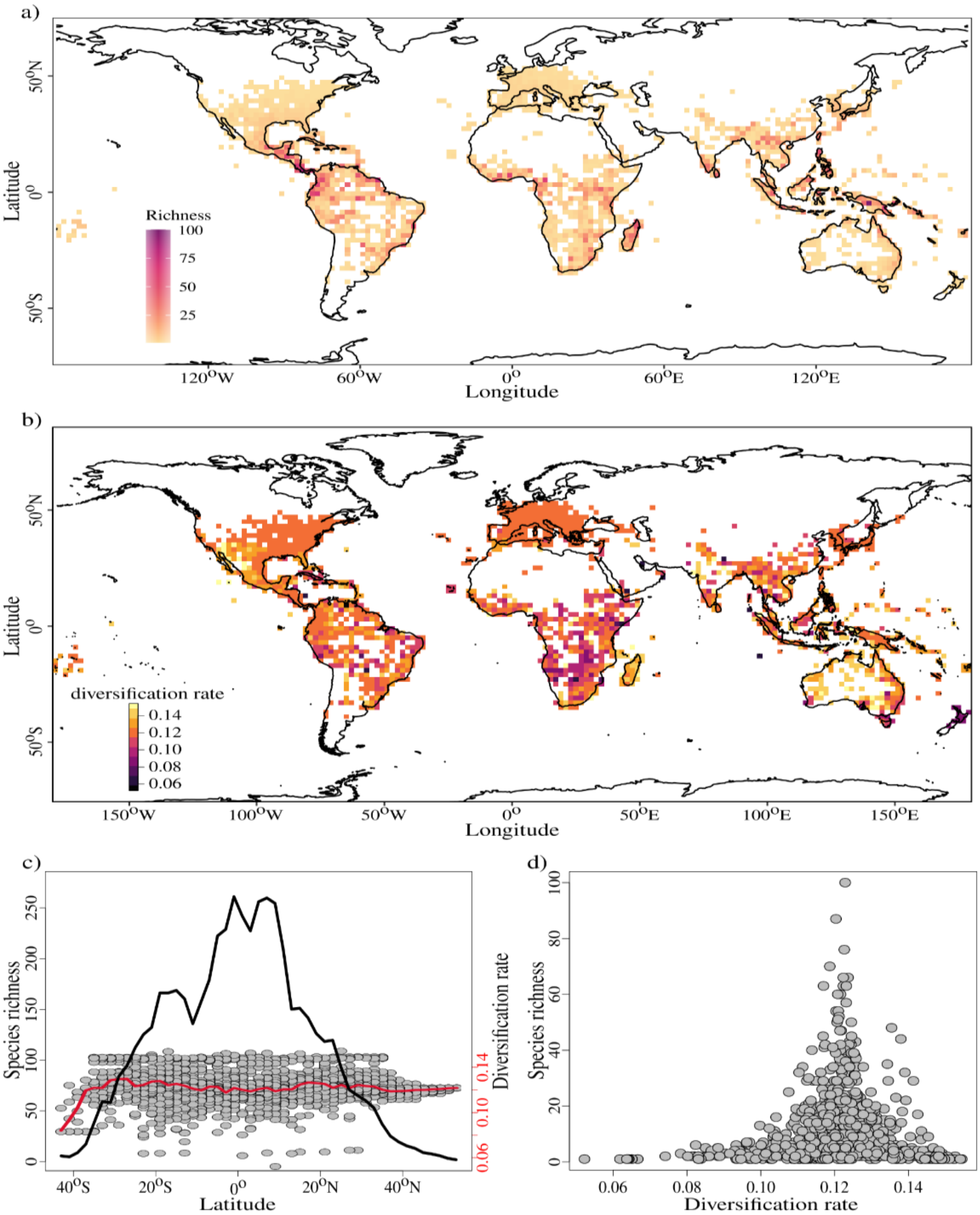

Figure 2. Spatial distribution of Ponerinae diversity. (a) species richness, (b) harmonic mean of diversification rate, based on 200 trees. Grid cell 2º x 2º. (c) latitudinal species gradient of Ponerinae (black line), with relatively homogeneous mean diversification rate along latitude (red line). Each gray dot represent the mean diversification rate per grid. (d) denotes the relationship between diversification and species richness by spatial cell (gray dots).

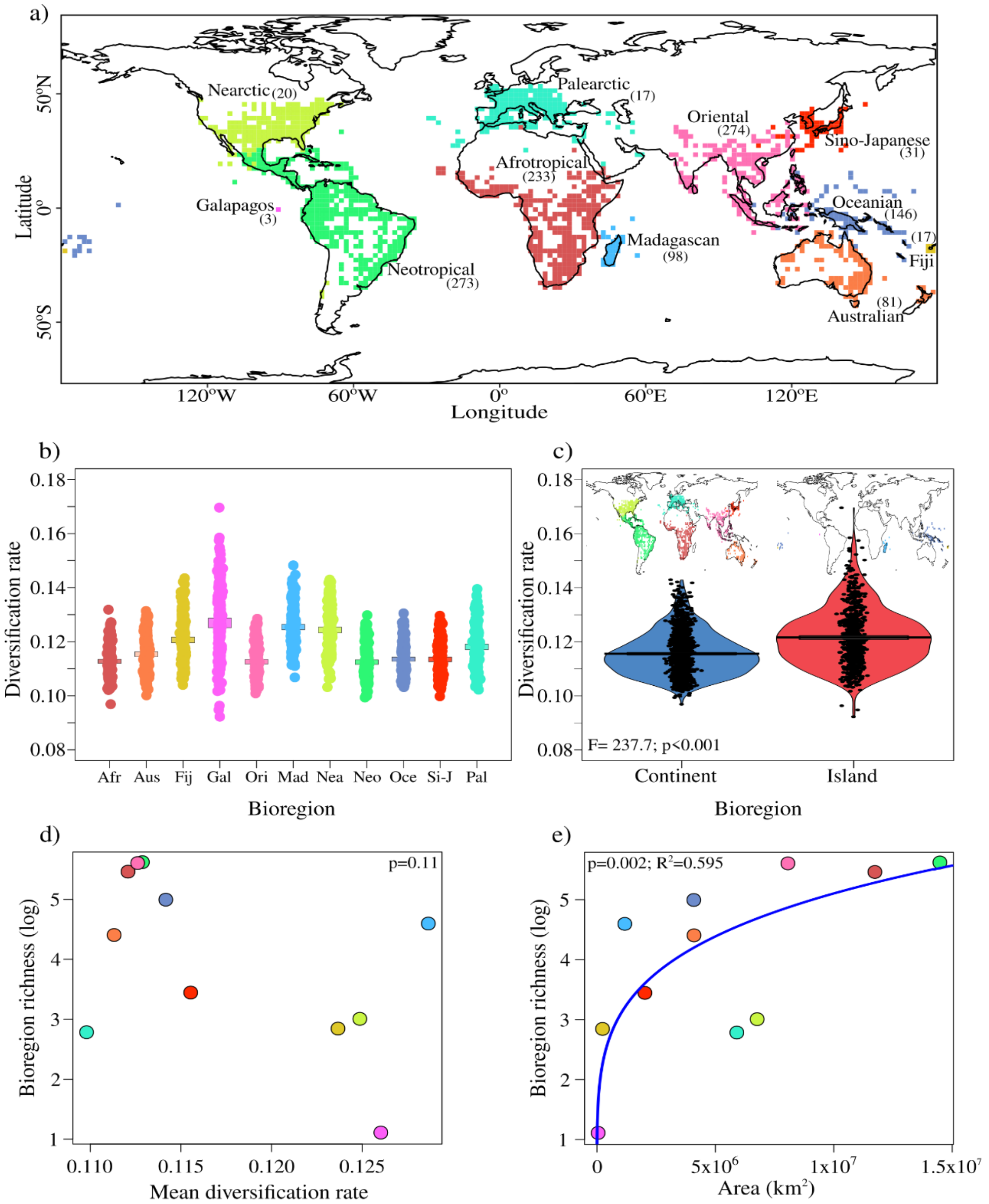

Figure 3. Ponerinae ant diversity and bioregions. (a) Bioregion delimitation based on Infomap analysis (species richness of each bioregion between parentheses). Bioregion nomenclature based on Holt et al. (2013). (b) variation in diversification rates across the eleven suggested bioregions, with each dot presenting the mean rate for each tree (200 trees). (c) archipelagian bioregions, on average, had higher diversification rates than continental or mainly continental bioregions. Regional richness is unrelated to diversification rate (d) but presents a non-linear relation with bioregion area (species-area relationship) (e).

Discussion

Historical Biogeography

A possible American origin for Ponerinae ants agrees with a previous biogeographic study by Moreau and Bell (2013), however, the authors used only 26 species. Thus, from both scenarios, Moreau and Bell (2013) and this study, the estimation of center of origin of Ponerinae remain relatively unreliable. In addition, we highlight the role of Indomalayan bioregion in early Ponerinae evolution. Most major internal clades of Ponerinae probably originated in the Indomalayan bioregion (Figure 1), and then dispersed mainly to Afrotropic and Australian bioregions. From that, the Afrotropics then became a center of origin of most genera in *Odontomachus* and *Plectroctena* groups. From the Afrotropics, the genera that eventually spread beyond the African limits became highly speciose, while nine endemic genera became poorly speciose (Figure 1 and Table S1). Such a scenario indicate that range expansion and transoceanic dispersal probably played an important role during the evolution of Ponerinae.

Other major center of origin and evolution of new lineages is the American continent, mainly the Neotropical region. The *Pachycondyla* group is the unique large Ponerinae clade restricted to one continent, the Americas. It is unclear if the group emerged and evolved solely in the American continent or if such ants also dispersed from the Indomalayan region. However, from a simple beginning, about 67 million years ago (Schmidt 2013), the *Pachycondyla* group became the dominant Neotropical Ponerinae, mainly in the Amazon Rainforest (Guénard et al., 2017).

Richness and diversification through space

Richness and diversification appear to be uncorrelated in Ponerinae ants. A clear latitudinal gradient in species richness is still present; notwithstanding the diversification of Ponerinae not presenting a relation with latitude. Except for the southernmost latitudes (New Zealand), diversification rates were relatively homogeneous along latitudes. Such latitudinal patterns of diversity agree with Economo et al., (2018), which also found a latitudinal species richness gradient but no significant variation in diversification rates along latitudes for all ant species.

The Ponerinae have possibly evolved with a spatial pattern similar to another hyperdiverse ant clade, *Pheidole* (Economo et al., 2019) and similar to the whole ant family (Economo et al 2018). The absence of systematic variation in diversification with latitude indicate that the latitudinal diversity gradient probably originates by a longer time for speciation in tropical regions than temperate ones, rather than variation in diversification across latitudes, as already pointed out by (Economo et al., 2018).

Bioregions and regional richness

The delimitation of bioregions for the Ponerinae follow a similar pattern to what was found, in a recent revision, for vertebrates (Holt et al., 2013). It includes a Neotropic/Nearctic separation, a continental Africa and Madagascar separation, and a Sino-Japanese bioregion, disconnected with the Palearctic (Holt et al., 2013). Such patterns may indicate climatic and geologic/geographic isolation features that act on vertebrate species (Holt et al., 2013; Ficetola et al., 2017), also drive ant distribution on a planetary scale. However, we found a more "permeable" Wallace line boundary, with the Oriental bioregion extending beyond Sulawesi Island, which usually belongs to the east side of the line.

The diversification rate proved to be a poor predictor of current Ponerinae diversity. There is no evidence that it influenced the richness along the latitudinal gradient (Figure 2c), local community assembly (Figure 2d), and, present variation among bioregions (Figure 3b) does not help explain regional richness either (Figure 3d). However, diversification plays a relevant role in islands/archipelagoes (Figure 3c), presenting higher diversification rates than continental lineages. It may indicate that isolate areas act as cradles of new ant species, mainly in the Madagascan bioregion (Figure 3b and d).

We are unable to infer if the high diversification rate on islands is due to a rapid increase in diversification post-colonization, a process previously documented in ants in Tropical Pacific Islands, for example (Sarnat and Moreau, 2011; Liu et al., 2020). Nevertheless, a similar process could be responsible for the high diversification rate of Pacific Ponerinae. At the same time, the Madagascan region presents the highest diversification rate across bioregions (Figure 3d). Ponerinae present ten non-closely related genera on Madagascar (Guénard et al., 2017), which suggests multiple colonization events on the island. Madagascar presents high relief and climate complexity, listed as the driver of the high diversity present on the island (Antonelli et al., 2022), including ants. For example, Camacho et al., (2021) also suggested that complex environmental variation there led to the emergence of a cradle of ant biodiversity. Thus, complex geographic and climatic features coupled with several colonization events could trigger an ideal scenario to increase Ponerinae diversification rates in this bioregion.

Area explains reasonably well the species richness across bioregions, as expected. Species-area relationships are known to describe ant diversity across islands (Zhao et al., 2020; Ohyama et al., 2021) and, according to our results, there are also positive species-area relationships across continents and islands. However, the Nearctic and Palearctic, the two bioregions with more discrepant biodiversity given the species-area model prediction (Figure 3e), present relatively big areas but are poor in species, probably in accordance with the latitudinal gradient of ant diversity (Economo et al., 2018). This indicates area alone does not explain richness along bioregions, but act jointly with latitudinal related features (for ants, long-term tropical stability, Economo et al., 2018).

In summary, our results suggest that large continental bioregion harbor more species while, proportionally, archipelagos bioregions are important centers of diversification of Ponerinae species.

Limitations

The lack of phylogenetic robustness at the species level hampers a fine-scale understanding of diversification along time and space for this group, which could bring a more reliable panorama of diversification in space. In addition, it impedes a fully reliable reconstruction of the direction and frequency of dispersal events over time. Such information could add better predictions about the role of colonization (e.g. age of first colonization and rate of colonization) on regional richness, which may explain current diversity patterns.

Due to the transcontinental distribution of several genera, probably manifold dispersal events within each genera occurred along Ponerinae evolution. Although some sources have sought to understand the historical biogeography of some specific genera (Matos-Maraví et al., 2018; Fernandes et al., 2021), a general and more accurate picture of Ponerinae dispersal routes remains unknown due to phylogenetic uncertainty within genera.

The available distribution data is relatively incomplete, lacking information on some species, which could also bias the results. Some probably speciose areas as the Amazon and African rainforests, and mainland Southeast Asia (Kass et al., 2022) remain relatively poorly sampled. However, current data on Ponerinae can still be very useful as a first purview into the past to understand ant diversification and, hence, the relevance of time and space for the evolution of clades with transcontinental distribution.

Concluding remarks

We found that the Indomalayan and then the American and Afrotropic bioregions acted as sources of species through Ponerinae geographic evolution since the Cretaceous. As a result, currently, Ponerinae presents a pantropical-centered distribution, with a secondary temperate distribution. The current distribution of Ponerinae follows a latitudinal species gradient where richness is uncorrelated with diversification rate.

When we tested Ponerinae distribution into bioregions, we found a high diversification rate in archipelagoes bioregions compared with continental ones, which highlights the relevance of islands as centers of origin for new Ponerinae ants. In addition, large continental landmasses present small diversification rates but harbor more Ponerinae species, in a clear species-area relationship along bioregions.

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Supplementary Information

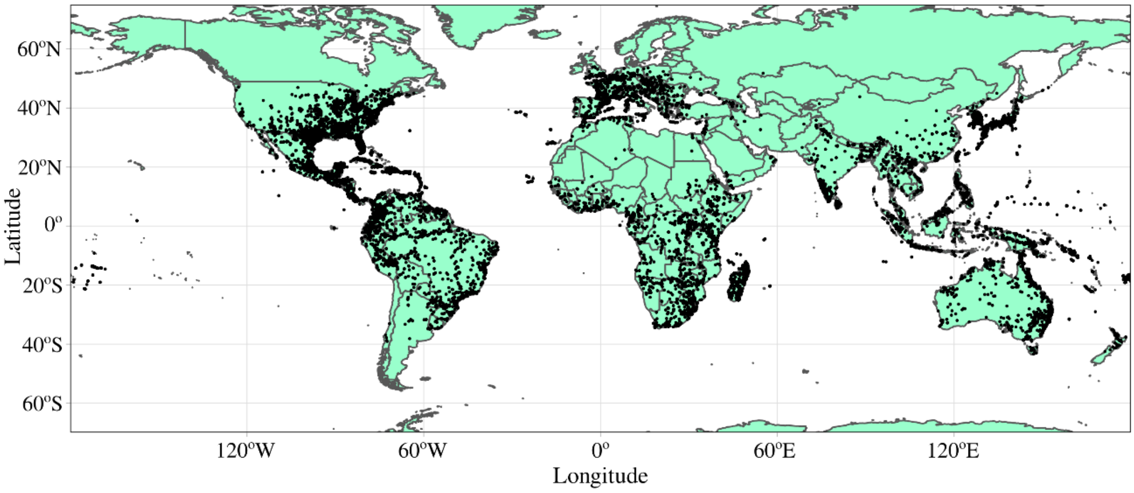

Figure S1. Distribution of 30,286 coordinates points for 1,065 Ponerinae species. Data from the Global Ant Biodiversity Informatics - GABI (Guénard et al., 2017).

Table S1. Genus richness and distribution across eleven bioregion used in this study. Occurrence data from Guénard et al., (2017) and genus richness from Bolton (2023). Af: Afrotropical, Au: Australian, Ne: Neotropical, Na: Nearctic, SJ: Sino-Japanese, Oc: Oceanian, Or: Oriental, Ma: Madagascan, Pa: Palearctic, Fi; Fiji and Ga: Galapagos.

Genus	Bioregions	Richness
Anochetus	Af, Au, Ne, Na, SJ, Oc, Or, Ma, Pa	115
Austroponera	Au	3
Bothroponera	Af, Ma,Or	43
Brachyponera	Af, SJ, Or, Oc, Au	18
Buniapone	Or	1
Centromyrmex	Ne, Af, Or	15
Cryptopone	Af, Au, Ne, Na, SJ, Oc, Or, Pa	23
Diacamma	Au, SJ, Or, Oc	44
Dinoponera	Ne	8
Ectomomyrmex	Au, Or, Oc, SJ	27

Emeryopone	Or, Pa	5
Euponera	Af, Ma, SJ,Or	25
Hagensia	Af	2
Harpegnathos	Or	9
Hypoponera	Af, Au, Ne, Na, SJ, Oc, Or, Ma, Fi, Ga, Pa	154
Leptogenys	Af, Au, Ne, Na, SJ, Oc, Or, Ma, Fi, Ga	316
Loboponera	Af	9
Mayaponera	Ne	7
Megaponera	Af	1
Mesoponera	Af, Au, Oc, Or, Ma, Pa	20
Myopias	Au, Or, Oc	46
Neoponera	Ne, Na	58
Odontomachus	8	73
Odontoponera	Oc, Or	2
Ophthalmopone	Af	5
Pachycondyla	Ne, Na	17
Paltothyreus	Af	1
Phrynoponera	Af	5
Platythyrea	Af, Au, Ne, Na, Oc, Or, Ma	40
Plectroctena	Af	16
Ponera	Au, Ne, Na, SJ, Oc, Or, Fi, Pa	59
Psalidomyrmex	Af	6
Pseudoneoponera	Au, Or, Oc	19
Pseudoponera	Ne, Au	6
Simopelta	Ne	22
Streblognathus	Af	2
Thaumatomyrmex	Ne	13

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PARTE II

A EVOLUÇÃO TEMPORAL DE PONERINAE

Capítulo 2

Manuscrito formatado nas normas da revista Evolutionary Biology

Unraveling the Ponerinae "paradox": Testing of the dynastic-succession hypothesis

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Abstract

Social complexity is thought to be positively correlated with species richness in ants. For this reason, Wilson and Höldobller (2005a) claimed that the evolution of Ponerinae ants is a "paradox": they comprise a large group with about 1.300 species, and yet they have simple social organization. The Dynastic-Successional Hypothesis (DSH) was proposed to explain the Ponerinae "paradox" as follows: Ponerinae rapidly diversified after the Cretaceous- Paleogene extinction event (K-Pg) until the Paleocene or early Eocene because of an increase in their prey diversity (arthropods) and lack of competitors. Then, the diversification was slowed down by a subsequent Myrmicinae radiation (more socially complex, better competitor ants). Using recent phylogenetic hypotheses for Ponerinae and diversification-through-time analyses considering phylogenetic uncertainty, we tested whether Ponerinae evolved as claimed by the DSH. Contrary to

DSH predictions, Ponerinae presented slow and gradual increase of diversification rate rather than an evolutionary radiation post-K-Pg event. In addition, contrary to DSH assumptions, we found no evidence of Myrmicinae radiation during Eocene. Our results indicate that a long, steady time for species accumulation, rather than an evolutionary radiation, drove the evolution of Ponerinae. The DSH is commonly used to explain the evolution of major ant clades, however, our work demonstrates that such evolutionary scenario is unlikely.

Key words: ant evolution, poneroid ants, evolutionary radiation, social complexity

Competing interests

None

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Introduction

Speciation and extinction ultimately determine species accumulation across the tree of life. In macroevolutionary studies, the diversification rate (speciation rate minus extinction rate) is commonly used to infer the net variation in the accumulation of new lineages through time (Helmstetter et al., 2021). In this sense, it is possible to measure when, in the past, a specific clade may have undergone either increases or decreases in diversification rate (Magallón & Castillo, 2009). Beyond inferring quantitatively how much diversification varies in time, we might also infer probable causes of such variation, or even, the causes of the absence of a variation, that is, when net diversification is constant through time (Mckenna & Farrell, 2006).

When a lineage presents fast increases in diversification rate over a short timescale, such events are called evolutionary radiations (Abe & Lieberman, 2009). Several factors may trigger evolutionary radiations, such as allopatric speciation triggered by geographic barriers (geographic radiation), a previously existing trait becoming advantageous and enhancing the speciation (exaptive radiation), or yet, through the emergence of an ecological factor that, even in sympatry, leads to an increase in both speciation rate and phenotypic disparification (Simões et al., 2016). Traits that facilitate diversification can also be considered a key innovation (Rabosky, 2017). These traits could be morphological, as the loss of flight muscles in beetles (Ikeda et al. 2012), sexually related, such as ornamentation in birds (Maia et al., 2013), or even group social behavior (Davis et al., 2009; Legendre & Condamine, 2018).

Ants are eusocial insects (Wilson & Hölldobler, 2005b) with a wide variation in social structure (e.g. colony size, caste polymorphism, mating system) across species (Ferguson-Gow et al., 2014). Such variation triggers, in some cases, changes in diversification rates. For example, Burchill and Moreau (2016) found that ant species with small colony sizes present a slower diversification rate than species with medium to large colony sizes, indicating the role of social structure in ant evolution. In this sense, Ponerinae ants present an intriguing relationship between social complexity and species richness. They form the third-largest ant subfamily, reaching about 1.300 species (Bolton, 2023). However, they have small colony sizes, usually dozens to a few hundred workers (Schmidt & Shattuck, 2014), low queen-worker dimorphism (Ito & Ohkawara, 1994), and some species are even queenless, with reproduction taken by workers (Viginier et al., 2004). Such a “simple” social and population structure leads Ponerinae to be considered a group with socially simple organization.

Because they break the coupling between low social complexity and high species richness, Wilson and Hölldobler (2005a) called the evolution of Ponerinae a paradox; in their words “globally successful yet socially primitive”. The authors sought to explain the paradox with the dynastic-succession hypothesis (DSH). This hypothesis claims that two of the largest tropical emergent ant groups, Ponerinae (less complex societies) and Myrmicinae (more complex societies), evolved under a successional diversification process. Firstly, Ponerinae would have experienced adaptive radiation after the Cretaceous-Paleogene (K-Pg) extinction, during Paleocene-Early Eocene, followed, in turn, by a Myrmicinae radiation (nowadays presenting 7.097 species (Bolton, 2023)), a superior competitor, which slowed down the diversification of Ponerinae during the Eocene (Wilson & Hölldobler 2005a). As arthropod predators, Ponerinae would have experienced an increase in diversification rate during the spread of tropical forests, post K-Pg extinction event, enabled by the occupying a larger niche volume during a small window of time (Wilson & Hölldobler, 2005a).

Here, we investigated the diversification through time of Ponerinae and Myrmicinae ants to test the support for the DSH. There are few alternative scenarios to

DSH considering different diversification histories of subclades of Ponerinae. One of them propose an early-burst-like evolution of the *Odontomachus* group (a large subclade of Ponerinae with about 400 species) about 40 million years ago (Schmidt, 2013) rather than and whole subfamily burst, as proposed by DSH. Thus, we also analyzed the diversification pattern of seven internal clades of this subfamily, partially based on the clade division by Schmidt and Shattuck (2014). In this sense, the analysis of the whole subfamily and the major internal groups enabled us to elucidate the diversification of Ponerinae through time and detect whether and when, in time, such ants experienced high diversification rates.

Materials and methods

Phylogenetic data

We used the recent and comprehensive phylogenies from Economo et al. (2018), encompassing 14.512 ant species and subspecies. From these ant trees, we extracted the 1.310 Ponerinae species and subspecies, which corresponds to about 91% of the described Ponerinae species (Bolton, 2023) and 7.192 Myrmecinae species and subspecies, corresponding to about 90.7% of Myrmecinae species (Bolton 2023).

Initially, Economo et al. (2018) built backbone phylogenies based on molecular data from 11 nuclear loci from 687 species, in which 90 belong to Ponerinae. We used here the trees based on Fossilized Birth- Death dating approach (Heath et al., 2014). The remaining species were drafted based on two methods of insertion based on backbone trees. The backbone trees were divided into clades (usually genera), where the species lacking from each genus were added. For the first method, missing species were randomly sampled starting in the crown age of each clade in a backbone tree. This means the crown age of a genus in a “complete tree” is the same age of such genus in the backbone tree.

For the second method, species insertion was made between the crown and stem age of the clade in the backbone tree. That is, the crown age of a genus in “complete trees” is older than in the first method (See Economo et al. (2018) supplementary material, for more details).

Following the author’s nomenclature, hereafter we refer to the phylogenies as either crown (developed with the first method) or stem (second method) phylogenies. Both crown and stem trees present uncertainty in age and phylogenetic position of lineages, mainly within each genera. The 200 phylogenetic trees were obtained from Dryad repository (doi.org/10.5061/dryad.g579t7k).

Diversification through time

To investigate the Ponerinae radiation claimed by Wilson and Hölldobler (2005a) we used two analytical approach. The first was the Bayesian Analysis of Macroevolutionary Mixtures-BAMM (v.2.5.0). BAMM uses a Bayesian approach to calculate a posterior estimate of variation in diversification rate through time enabling to draw the probable diversification scenario through the history of a clade. After running the BAMM software, the output was analyzed in the R software (R Core Team, 2020), with the *BAMMtools* package (Rabosky et al., 2014). The Ponerinae trees are relatively large (1.310 species and subspecies), which, in turn, would consume several weeks to run the diversification for each of 200 trees with our computational power. Thus, we select randomly 30 trees, 15 stem and 15 crown trees, to test our evolutionary hypothesis. Based on our results, we argue that the 30 phylogenetic trees shows a reliable diversification scenario of Ponerinae ants.

To each tree, we ran 20 million generations with Markov Chain Monte Carlo approach. We discard the first 25% of generations as burn-in. We also assume a conservative *globalSamplingFraction* = 0.8, (there is, 80% of described Ponerinae species are present in phylogenetic trees) and chose an *expectedNumberOfShifts*=30.

Beyond Ponerinae as a whole, we also analyzed the major clades of Ponerinae separately. Based on Economo et al. (2018) trees, we divided the subfamily into seven internal clades (Figure S1), partially based on a previous division by Schmidt and Shattuck (2014). Nominally, *Plathytyrea* (Pl clade- 39 spp), *Pachycondyla* genus group (PaG clade- 119 spp), *Ponera* genus group (PoG clade- 173 spp), *Plectroctena* genus group (Pl clade- 59 spp), *Hypoponera* (Hy clade- 170 spp), *Leptogenys* (Le clade- 332 spp). Due to the uncertainty in the position of some genera (e.g. *Myopias* and *Brachyponera*), the *Odontomachus* genus group (OG clade) may present between 397 and 317 species, depending on tree; in most of the trees analyzed it presented 377 species. See Figure S1 to a graphical resolution of the OG clade.

To test the second claim of Wilson and Hölldobler (2005a), that Myrmicinae undergo a radiation during Eocene, we also run the BAMM to Myrmicinae trees. Due to high computational demanding (more than 7000 species), we chose 20 Myrmecinae (10 stem and 10 crown) trees to rum the BAMM analysis, with 20 million generations, choosing a *globalSamplingFraction* = 0.8, and *expectedNumberofShifts*=100.

BAMM was criticized due to the high sensibility of the prior expected number of shifts (Moore et al., 2016, but see Rabosky et al., 2017), resulting in a biased posterior estimate. To cross-validate our results, we also estimate the net diversification rate through time from Compound process on Mass Extinction Times (CoMET) (May et al. 2016) analysis from TESS package (Höhna et al. 2016). To the same tree run in BAMM, we run 20 million iterations, discarding the first 25% of generation as burn-in. We assume a conservative *samplingProbability* = 0.8. The other parameters remained as the default.

We also run the CoMET analysis to internal Ponerinae clades, considering the same clade division that in BAMM.

We also run the CoMET analysis to 30 Myrmicinae trees (15 stem and 15 crown) using the same parameters from Ponerinae, with 20 million generation and *samplingProbability* = 0.8.

Results

We found a lack of support for an increase in the diversification rate of Ponerinae after the K-Pg event (Figure 1 and Figure 4a and 4b). Ponerinae presents a steady increase in diversification rate through time, until about 20 million years, when diversification seems to increase. Such increase is sharper in crown trees than in stem trees (Figure 1b, 1c and Figure 4a, 4b), which indicate that the recent increase could be, in some degree, result from phylogenetic uncertainty rather than an evolutionary event.

The variation in diversification rates through time for internal Ponerinae clades (Figure 2 and Figure S2) was greater in some cases, as *Leptogenys* (Figure 2h, o). For stem trees, the recent evolutionary radiation of Ponerinae is mainly the product of a fast increase in the diversification of the OG clade (Figure 2g) while for crown trees both OG and PoG clades had a generally fast increase in diversification rates (Figure 2k and n).

From BAMM, the internal Ponerinae clades showed mean net diversification rates varying from 0.04 (*Platythyrea*) to 0.11 (*Leptogenys*), with sensible differences between crown and stem trees (paired t test, *p*= 0,007). In sum, the high variation in diversification rate across internal clades from both BAMM (Figure 2) and CoMET (Figure S2) makes it unreliable to describe a scenario of evolution of most internal major clades, even if more trees were considered.

From BAMM, the stem Myrmicinae trees present a slow and continuous increase of diversification until near present, when diversification increase (Figure 3b). The crown trees (Figure 3c) present a more constant diversification from late Cretaceous to mid-Eocene, followed by a constant increase in diversification rate during last 30 million years. On the other hand, CoMET analysis (Figure 4c and 4d) shows a decrease in diversification rate during Eocene-Oligocene (about 20-60 million years), contrasting with DSH assumptions. However, both analysis show an increase in diversification rate during the last 20 million years, to Myrmicinae ants.

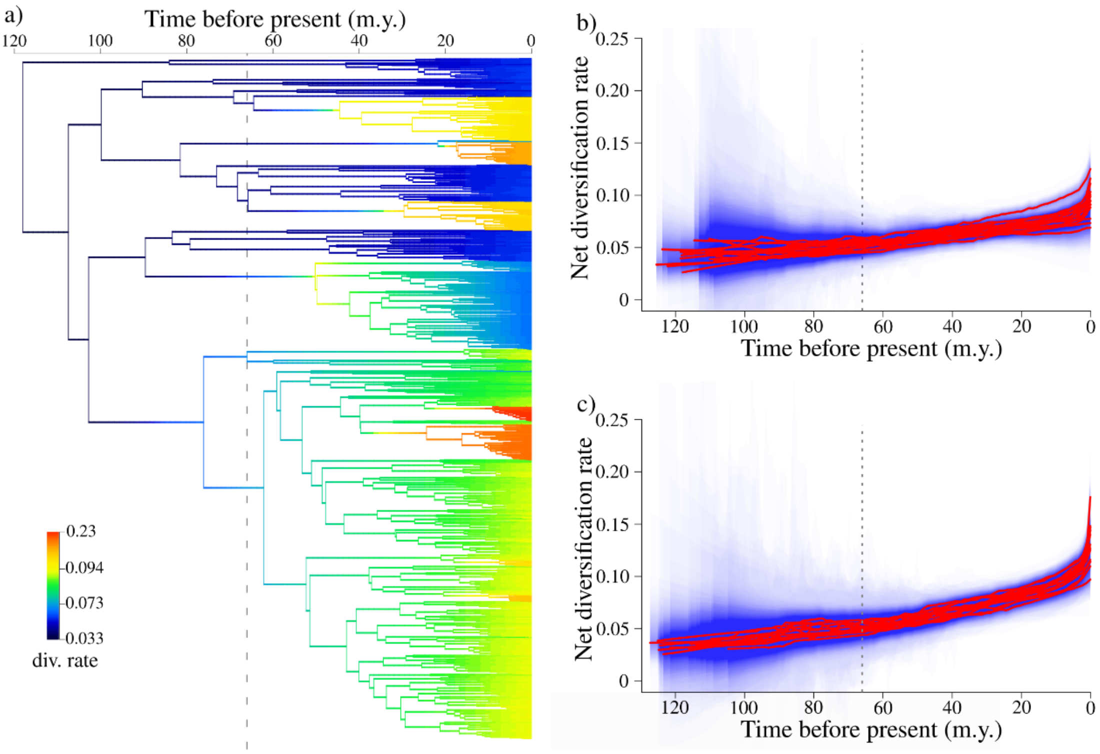

Figure 1. Diversification rate through time for the Ponerinae subfamily. In (a), the diversification rate in one sampled tree, just for visualization purposes. In (b) diversification through time for 15 stem trees, and in (c) for 15 crown trees. Vertical dashed line denotes K-Pg boundary about 66 million years.

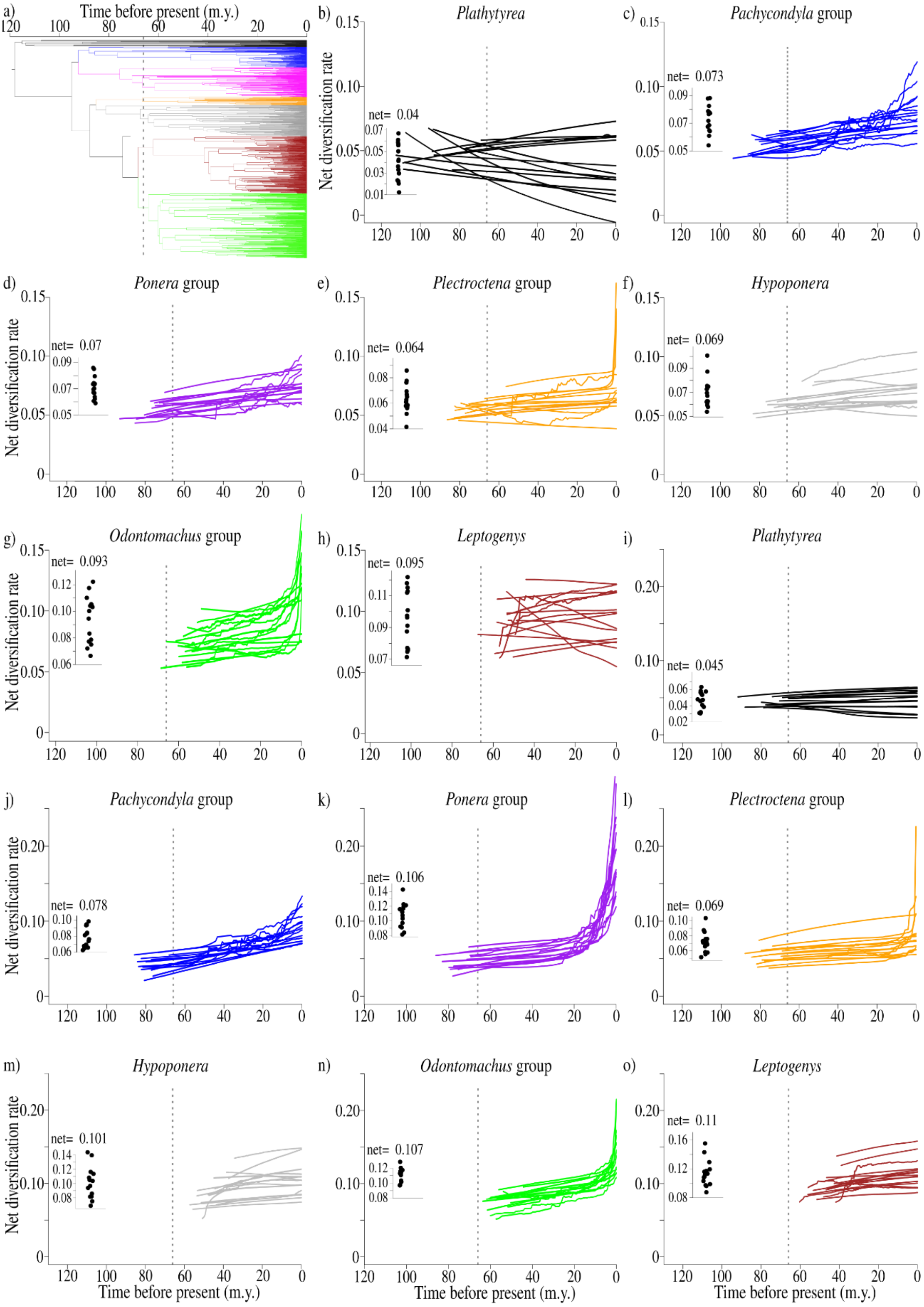

Figure 2. Diversification scenario for internal major clades of Ponerinae based on 15 stem trees (b-h) and 15 crown trees (i-o). The phylogeny in (a) shows colors for each of the seven internal clades analyzed (b-h). The phylogenetic uncertainty was higher in some

clades than others (e.g. *Leptogenys* and *Hypoponera*- f,h,m). The recent increase of diversification of Ponerinae seems to have been driven mainly by *Odontomachus* and *Ponera* groups (g,k,n). The dots in the inset chart show the mean diversification rate in each tree and net is the average to 15 trees. Vertical dashed line denotes K-Pg boundary about 66 million years.

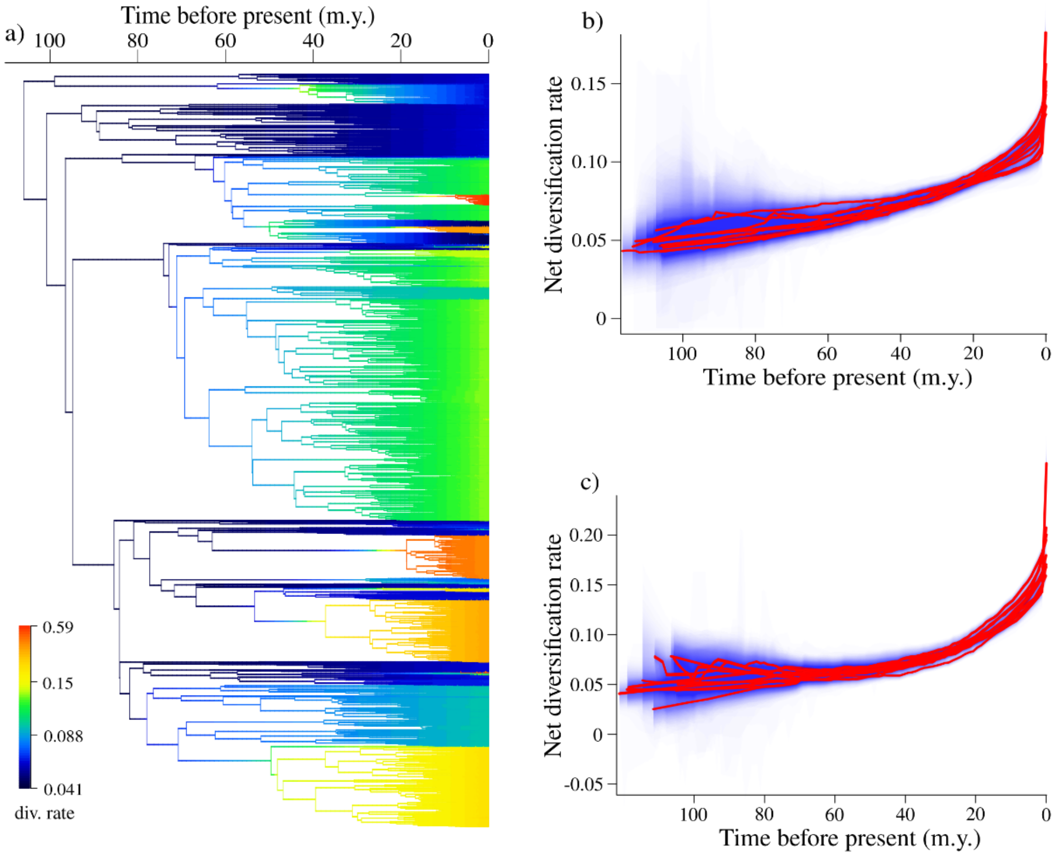

Figure 3. Diversification rate through time for the Myrmicinae subfamily. In (a), the diversification rate in one sampled tree, just for visualization purposes. In (b) diversification through time for 10 stem trees and, in (c) for 10 crown trees.

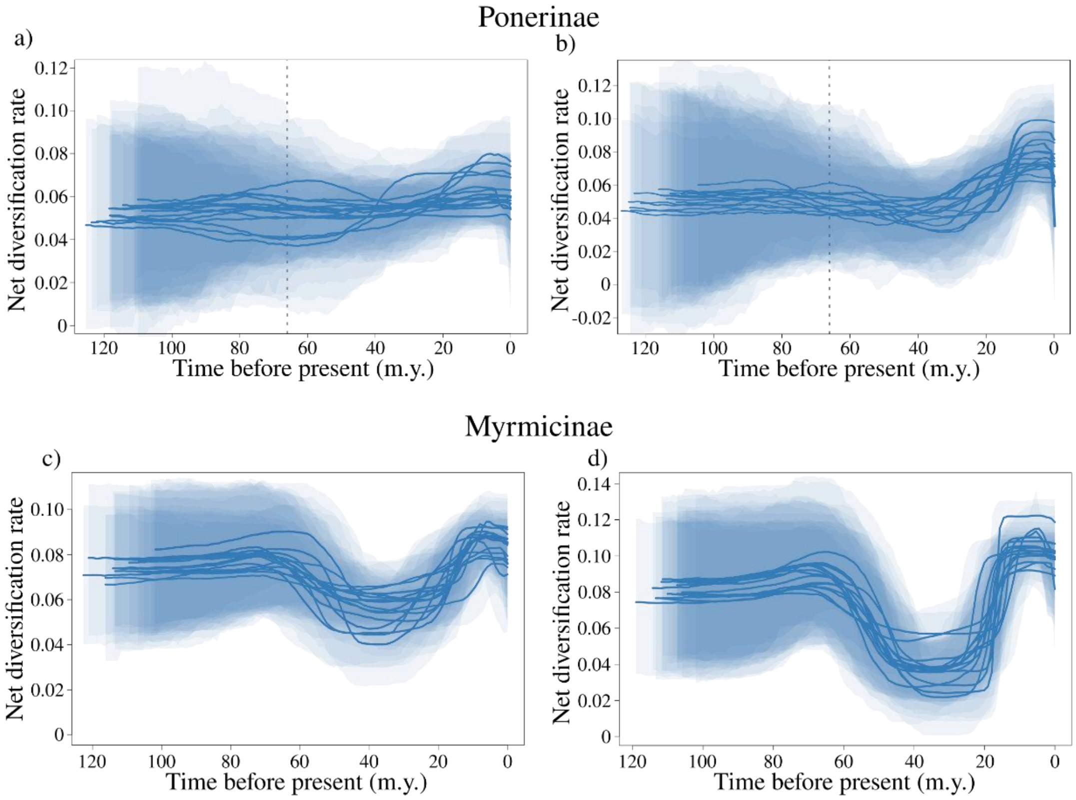

Figure 4. Diversification rate trough time from CoMET analysis. Ponerinae diversification based on 15 stem (a) and 15 crown (b) trees. Vertical dashed line denotes K-Pg boundary about 66 million years. Bellow, the diversification to Myrmicinae, based on 15 stem trees (c) and 15 crown trees (d).

Discussion

We reject the DSH as an explanation for the “Ponerinae paradox”. Our results contrast sharply with the first phase of the DSH, as suggested by Wilson and Hölldobler (2005a), which proposes an evolutionary radiation of the Ponerinae after the K-Pg extinction event. Ponerinae lineages do not present evidence of an evolutionary radiation post-K-Pg event from our analyses. The answer to the “Ponerinae paradox” involves a continuous increase in diversification rate followed by a more abrupt increase in diversification near the present (Figure 1b and c). Such an increase in diversification rate toward the present was not homogenous across Ponerinae but results mostly from increases in the OG clade (Figure 2g and n) and to a lesser extent in the PoG clade (Figure 2k). The internal clades had a large variation in diversification rate across the 30 trees tested, demonstrating a degree of phylogenetic uncertainty in results; however, general aspects of Ponerinae diversification were consistently found regardless of the uncertainty.

Some biogeographic and ecosystem prerequisites that would have triggered the successional dynasty, probably occurred. There is evidence of an increase in the Neotropical forest recovery a few million years after the K-Pg event (Carvalho et al., 2021), which, in turn, could help sustain an elevated arthropod richness (Wardhaugh, 2014). However, we found no evidence of Myrmicinae radiation during Eocene, but a possible increase of diversification near the present. In addition, based on fossil records, some authors suggest that the competition between Ponerinae and Myrmicinae was weaker than Wilson and Hölldobler (2005a) assumed. Dlussky and Wedmann (2012) argue that most fossils of Myrmicinae were found in ambers, which may indicate that such a subfamily inhabited preferentially the arboreal strata. On the other hand, Ponerinae evolved preferentially on soil (Lucky et al., 2013), leading to a spatial niche portioning, reducing a possible effect of competition (Dlussky & Wedmann, 2012). Taken together, whichever biotic and abiotic prerequisites were present after the K-Pg event to lead to Ponerinae radiation, seems to not have greatly influenced the diversification of such ants.

Beyond DSH, other features than niche and competition could answer the "Ponerinae paradox". The slow and continuous increase in the diversification rate along most of Ponerinae history suggests the role of time to explain the nowadays richness of the subfamily. Considering all ants, Economo et al. (2018) demonstrated that tropical ant lineages are older than extratropical ones and time explains the ant latitudinal gradient of diversity rather than variation in diversification rate. Ponerinae presents primarily a pantropical distribution (Schmidt, 2013), which suggests that the time for species accumulation was a relevant driver of Ponerinae richness. Other plausible features include historical biogeography: Ponerinae presents several dispersal events across continents, with the largest genera (e.g. *Leptogenys* and *Hypoponera*) having transcontinental distributions, while small genera present restrict distributions (Schmidt & Shattuck, 2014). The conceptual correlation could indicate the support of colonization frequency and/or species-area relationship to Ponerinae diversity. The rate and age of colonization across bioregions, known to influence the regional richness (Kennedy et al., 2017; Liu et al., 2021), remains unexplored for Ponerinae ants.

In addition, phylogenetic uncertainty may partially explain the acceleration of diversification rates toward the present. Small genera such as *Euponera*, *Mesoponera*, *Paltothyreus* (OG clade), and *Myopias* (Figure S3 and S4) present high net diversification rates (> 0.5) in some trees. This, in turn, leads to sensible differences in the increase of diversification rate near present in some but not all trees. Such differences suggest a degree of phylogenetic uncertainty in the recent Ponerinae radiation. This indicates that, for example, the recent evolutionary radiation in the OG clade could be a phylogenetic artifact. Such great variation in diversification rates impedes the reliable testing of the diversification scenario of major internal clades, as proposed early-burst of *Odontomachus* genus group (Schmidt, 2013).

Concluding remarks

We demonstrated that the dynastic succession hypothesis, which is often used to explain the diversification of ants after K-Pg. extinction, is not valid for Ponerinae evolution. Both Ponerinae and Myrmecinae does not present a successional evolutionary radiation, as predicted by DSH. The continuous and slow increase in the diversification rate over more than 100 million years suggests a role of time to explain the “Ponerinae paradox”. In addition, Myrmicinae also does not present an evolutionary radiation during Eocene. In summary, even presenting a simple social organization and being considered “primitive” ants, Ponerinae diversity flourished, maintaining an increasing and continuous diversification since the mid-Cretaceous.

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Supplementary information

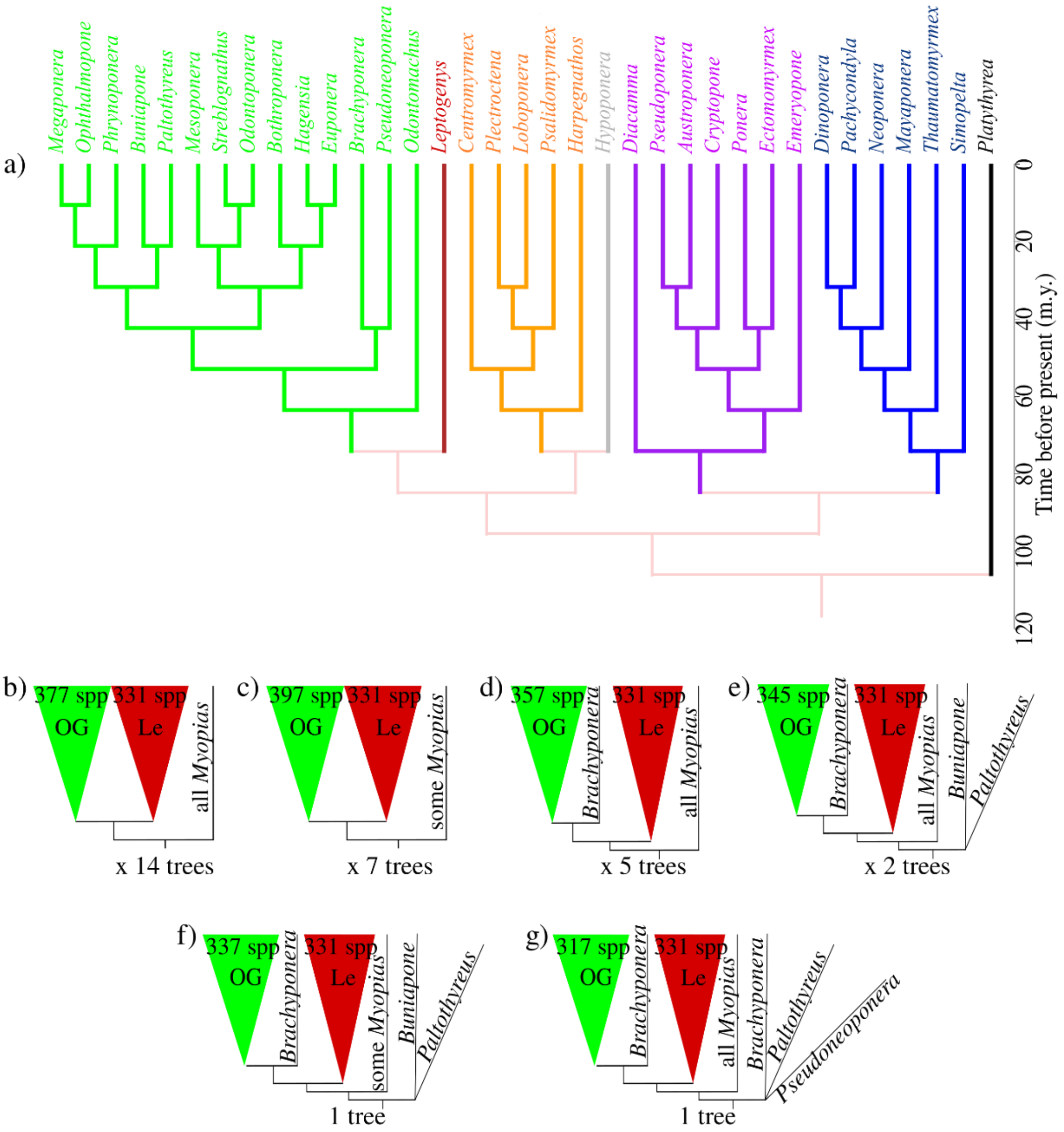

Figure S1. In (a), genus-level phylogeny of Ponerinae, indicating, by colors, the genus or genera that compose each internal major clade (Figure 2, in main text). Due to polyphyly of sister genera, *Anochetus* and *Odontomachus*, the *Odontomachus* lineage here, represent both genera. Green= *Odontomachus* genus group, brown= *Leptogenys*, orange= *Plectroctena* genus group, gray= *Hypoponera*, purple= *Ponera* genus group, blue= *Pachycondyla* genus group, black= *Plathytyrea*. To b-g, represent the simplified draft of uncertainty in relationship between OG and *Leptogenys* clade with external groups, black branches, not considered in diversification scenario of OG neither *Leptogenys* clade. Phylogeny from Economo et al. (2018).

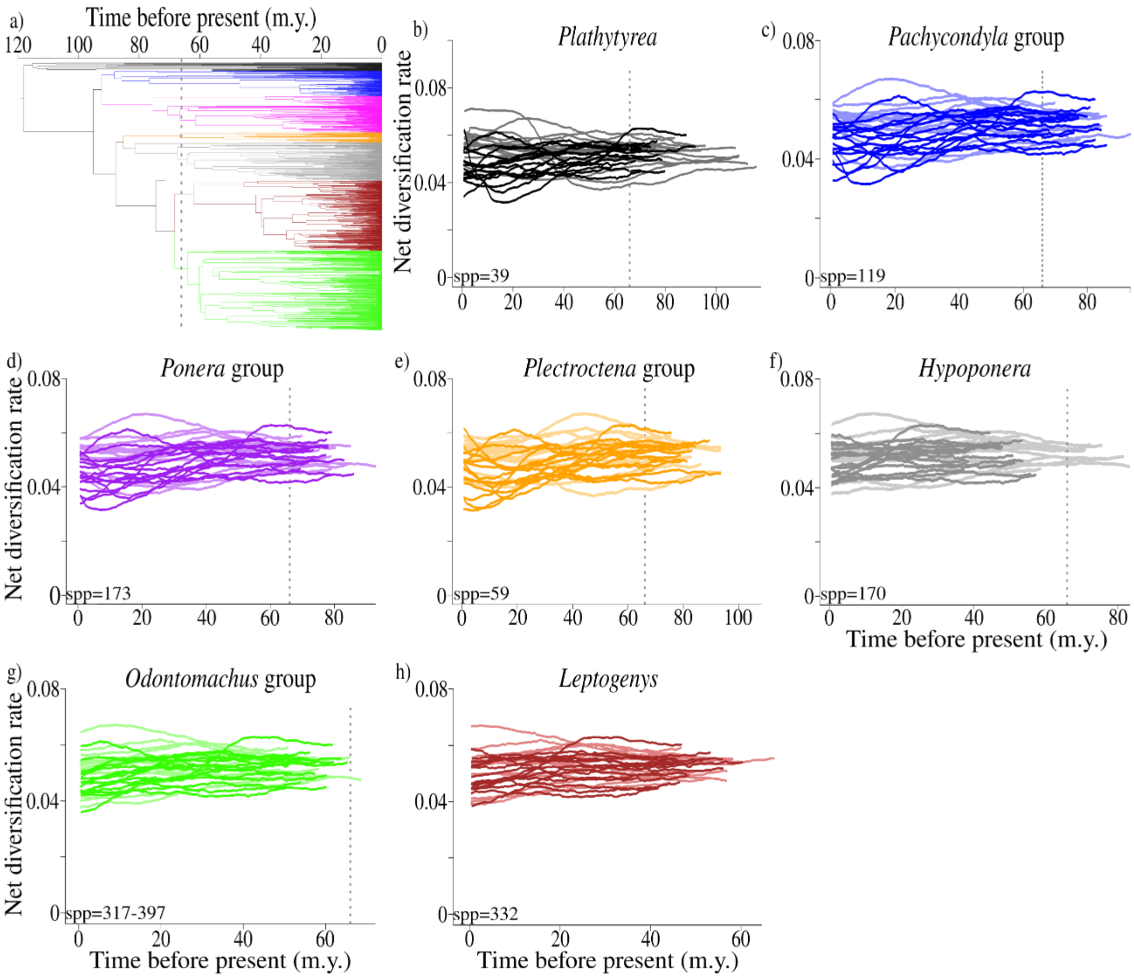

Figure S2. Diversification through time to internal Ponerinae clades based on CoMET analysis. In (a) a sampled tree with branches painted illustrating each of seven internal clades (b-h). Each line in (b-h) represent one phylogenetic tree. Darker lines show crown trees and lighter lines, stem trees. Dashed vertical line denotes the Cretaceous-Paleogene extinction event.

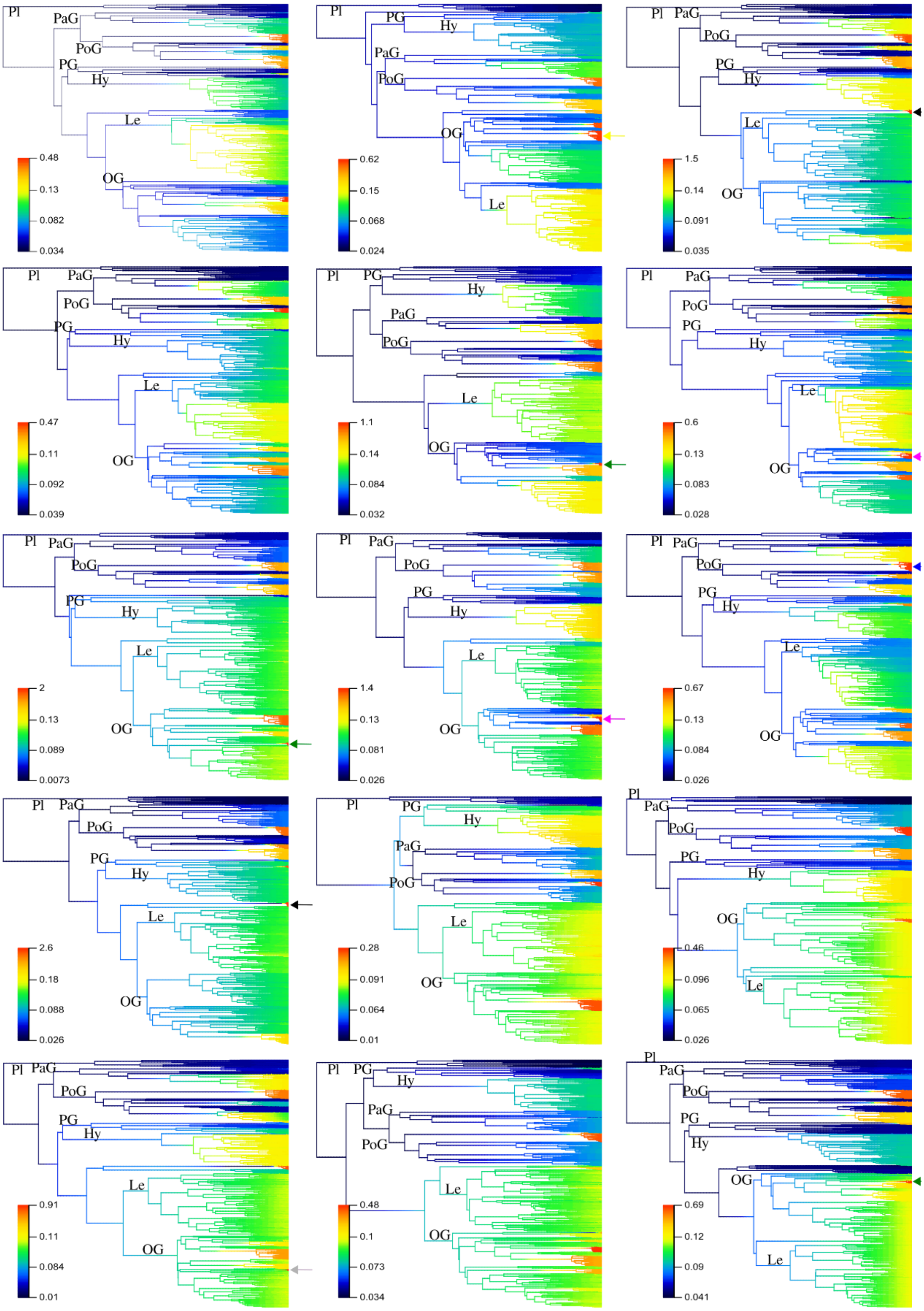

Figure S3. Diversification rate to each of 15 crown trees of Ponerinae. To trees that present very high rate (>0.5), the clade with highest rate are highlighted by arrow, *Myopias* (black), *Euponera* (purple), *Mesoponera* (green), *Paltothyreus* (gray), *Diacamma* (darkblue), *Bothroponera* (yellow). Pl= *Plathytyrea*, PaG= *Pachycondyla* genus group, PoG= *Ponera* genus group, PG= *Plectroctena* genus group, Hy= *Hypoponera*, OG= *Odontomachus* genus group, Le=*Leptogenys*. Color bar indicate the net diversification rate.

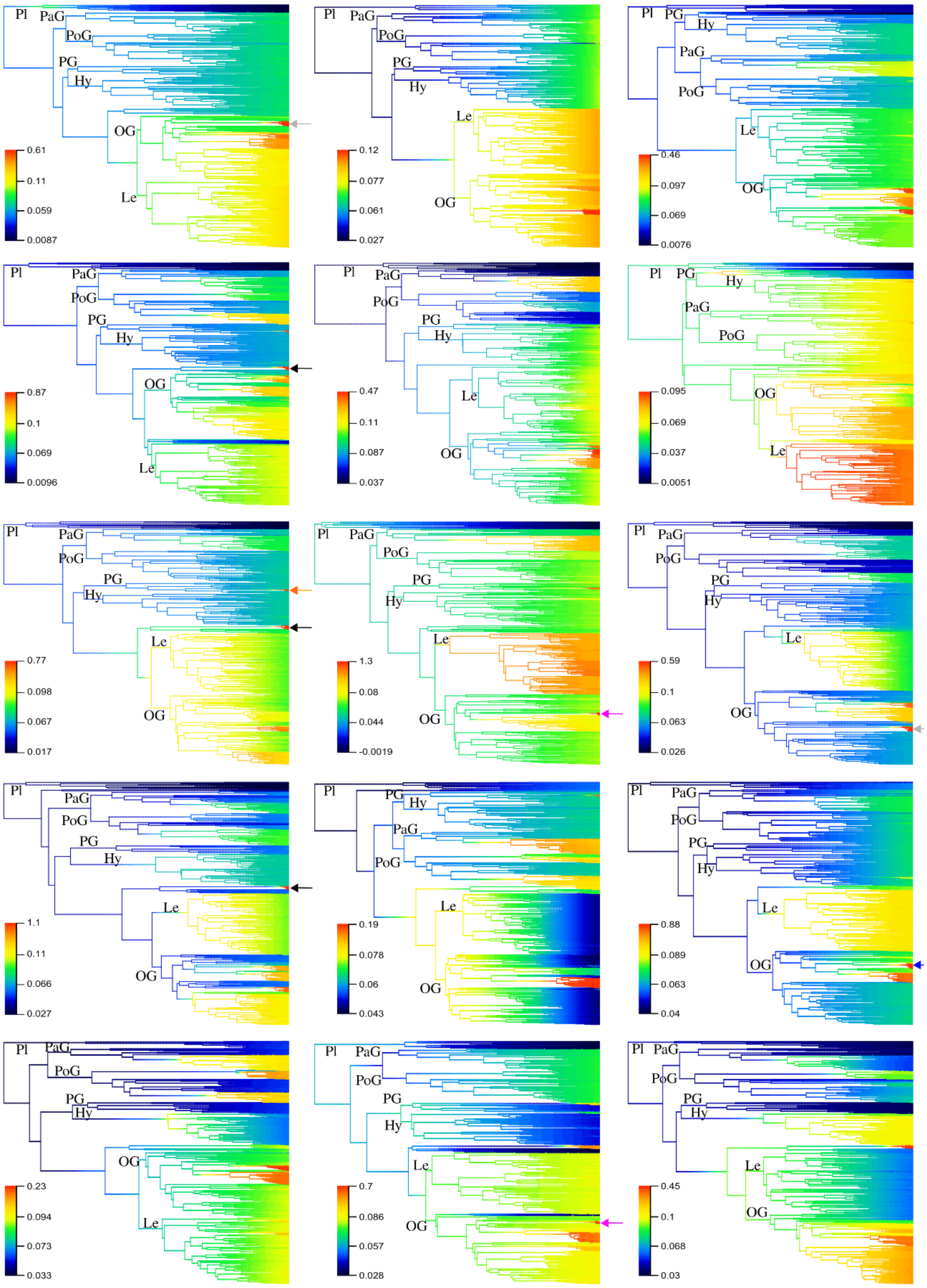

Figure S4. Diversification rate to each of 15 stem trees of Ponerinae. To trees that present very high rate (>0.5), the clade with highest rate are highlighted by arrow; *Myopias* (black), *Euponera* (blue), *Mesoponera* (purple), *Pseudoneoponera* (gray), and, *Loboponera* (orange). Pl= *Plathytyrea*, PaG= *Pachycondyla* genus group, PoG= *Ponera* genus group, PG= *Plectroctena* genus group, Hy= *Hypoponera*, OG= *Odontomachus* genus group, Le=*Leptogenys*. Color bar indicate the net diversification rate.

PARTE III

A REPRODUÇÃO POR OPERÁRIAS EM PONERINAE

Capítulo 3

Manuscrito formatado nas normas da revista Journal of Evolutionary Biology

Macroevolutionary consequences of reproduction by ant workers

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Conflict of interests

None.

Abstract

The mode of reproduction affects the long-term probability of persistence of a species. In extreme cases, it could negatively affect the evolution of lineages and lead to evolutionary dead-ends - negative diversification and irreversible transition from a dead-end reproduction mode to an alternative reproduction mode. In some Ponerinae ants, reproduction is not by a queen but by a fertile worker called a gamergate. Although the gamergate has appeared several times in the Ponerinae, it comprises only about 6% of the Ponerinae species, making it a candidate for an evolutionary dead-end. To test the dead-end scenario, we ran Hidden State Speciation and Extinction (HiSSE) models over 200 Ponerinae phylogenies. Based on currently knowledge about gamergate, we found no evidence that reproduction by worker lead to an evolutionary dead end. Models that consider an irreversible transition from gamergate to non-gamergate presented bad fit. In addition, we found no evidence of negative diversification to lineages with gamergate trait. Both assumptions of evolutionary dead end were not found in gamergate evolution. Our work shed light the macroevolutionary effects of two distinct reproductive castes on social insects suggesting that the reproduction by workers does not seems to trigger high extinction rate along ant evolution.

Keywords: Gamergate, evolutionary dead end, fertile workers, ant diversification.

Introduction

The role of reproduction in macroevolution has long been considered crucial. East (1918) was one of the first to outline the importance of reproduction in evolutionary process, due to maintaining mechanisms that generate variation among individuals, a central aspect of natural selection. Since then, with the development of modern phylogenetic comparative methods, we are now able to infer the role of reproduction at the macroevolutionary level (Juri et al. 2018). It has been discovered that some reproductive modes can actually negatively influence species diversification (speciation minus extinction). For example, studies have uncovered a negative influence of specialization in flower pollination on diversification in *Ruellia* plants (Tripp and Manos 2008). In addition, self-fertilization in plants (Day et al. 2016) and reproductive isolation in small populations (Brumm et al. 2010) could also reduce the long-term probability of species survival.

Thus, key reproductive traits (e.g., those leading to a particular mode of reproduction) that negatively affect diversification rates can lead to evolutionary dead-ends (Vamosi et al. 2014). Broadly speaking, evolutionary dead-end traits can be defined as those that reduce capacity for lineage persistence and/or diversification (Day et al. 2016). However, here, we follow Takebayashi and Morrell (2001), who proposed a more restricted definition of an evolutionary dead-end in which a trait leads to a negative net diversification rate and the lineages cannot revert the trait that lead to dead end. In this case, a trait may be beneficial on shorter time scales, which explains why such a trait exists, but not over longer periods, resulting in higher extinction than speciation rates (Agnarsson et al. 2006) and thus, to an dead end.

Ants exhibit a variety of morphological castes, including queens, majors, and workers (Trible and Kronauer 2017). Normally, only the queen and male breeds (Heinze and Tsuji 1995), however, in some species, workers exhibit reproductive activity, possessing a viable spermatheca that allows the deposition of fertile eggs. Such fertile workers are referred to as gamergate (Blatrix and Jaisson 2000). The gamergate (hereafter considered a reproductive trait) is spread across at least five ant subfamilies: Amblyoponinae, Ectatomminae, Myrmeciinae, Myrmicinae, and Ponerinae (Dietemann et al. 2004; Monnin and Peeters 2008; Taylor and Alpert 2016). Nevertheless, most of gamergate are present in Ponerinae, where phylogenetic relationships suggest multiple independent origins of gamergate within Ponerinae (Blatrix and Jaisson 2000).

Despite the numerous independent origins, species with the gamergate trait account for only about 6% of all Ponerinae species (table S1), which may indicate low diversification success compared to have a queen as the protagonist of reproduction. Low dispersal ability, probably due to dispersal by foot, once worker do not have wings, and low genetic diversity are known to be associated with gamergate trait (Tay and Crozier 2000; Viginier et al. 2004). Also, fertile workers probably presents lower fecundity compared to queens (Peeters et al. 2000; Peeters and Ito 2001; Monnin and Peeters 2008), which may result in smaller colonies.

Although fertile workers could coexist with queen in some species (Monnin and Peeters 2008), the presence of such workers can still affect the fitness of colony. For example, Liebig and Poethke (2004) found that colonies with reproduction by fertile workers present four times more mortality rate that colonies with reproduction by queen in *Harpegnathos saltator*. In addition, the egg-laying rate by worker was smaller than by queen to same species *H. saltator* (Peeters et al. 2000). Unfortunately, more detailed data about coexistence between such workers and queen are scarce, and here we assume that even coexisting, fertile workers could affect the species evolution in a similar way that species with reproduction only by workers. Thus, taken together, such eco-evolutionary features suggest that reproduction by fertile workers could hinder the persistence of such species and explain why reproduction by workers constitute the minority of Ponerinae ants. Hence, we investigate whether the advent of reproduction by workers leads to an evolutionarily dead end in Ponerinae ants.

Materials and Methods

Data collection

Phylogenetic data were obtained from the most comprehensive ant phylogenies published by Economo et al. (2018), covering more than 14,500 ant species, and alternative phylogenetic hypotheses were considered. The authors generated backbone phylogenies with molecular data for 687 ant species from 269 genera, forming 262 terminal clades. The insertion of the remaining species was designed using two approaches, referred to as "crown" and "stem" drafting (hereafter crown and stem trees, respectively) by Economo et al. (2018). For crown trees, the crown age of each terminal clade in the backbone trees (usually genera) was taken as the crown age of the grafted clades in "complete" trees and the edge length was scaled from this age. In stem trees, however, the crown age of the grafted clade is a random age between the crown age and the stem age of the backbone, and the edge length was scaled from this age. The 200 trees were obtained from Dryad repository (doi.org/10.5061/dryad.g579t7k). For more details on tree establishment methods, see (Economo et al. 2018).

Such trees include 1,160 Ponerinae species (removing subspecies and invalid species), about 91% of the described Ponerinae species (Bolton 2023). The Ponerinae trees have uncertainties in branch lengths and topology, so using a single phylogenetic hypothesis can lead to misleading results (Rangel et al. 2015). Therefore, we used the one hundred available phylogenetic hypotheses for crowns and stems trees, accounting for variation in age and relationships among species.

We obtained a list of Ponerinae species with the gamergate trait from literature, searching for terms “gamergate AND ants”, “gamergate AND Ponerinae”, “gamergate AND reproduction” (Table S1). Due to a lack of data on the number of gamergate (one or more per colony) in several species, we divided species into two categories: Presence of a fertile worker (gamergate - either one or more individuals) or absence of a fertile worker (reproduction is an exclusivity of the queen ant). Thus, from 1,160 species, 65 of them present gamergate, while 1,095 species did not (Figure 1). This information was used to create a binary variable for the presence or absence of the Gamergate trait for the list of Ponerinae species.

State-Dependent Diversification analysis

To investigate whether the gamergate trait leads to an evolutionary dead end, we ran HiSSE (Hidden State Speciation and Extinction) models to estimate speciation, extinction, and transition rates for each trait state (Beaulieu and O'Meara 2016). HiSSE allows inferences to be made about whether diversification rates are due to the effect of a measured trait or whether an unmeasured trait (hidden) could also be influencing diversification (Beaulieu and O'Meara 2016). In sum, the HiSSE model assume that related with an observed trait, there is an unmeasured trait that can potentially generate a different evolutionary dynamics than generated by observed trait (Beaulieu and O'Meara 2016). Such an approach reduces the probability of Type I error (O'Meara and Beaulieu 2016) and thus reduces the probability of error in inferring the effect of a particular characteristic on diversification rates.

Thus, for each state (gamergate absent or present), there are two alternative states, A (when hidden state is not considered) or B (when hidden state is considered), resulting in four possible states (0A, 1A, 0B, 1B). Because we want to determine whether the gamergate trait affects Ponerinae diversification, we suppressed the hidden state for non-gamergate species (0B state) and retained the unmeasured state for species with the trait (1A and 1B). HiSSE uses maximum likelihood inference to estimate the parameters τ(tau)= speciation (λ) + extinction (μ), extinction proportion, $\varepsilon = \mu/\lambda$, and transition (q) from one state to another and vice versa. To access extinction and speciation rates separately and then calculate diversification rate, we use equations S1a and S1b of (Beaulieu and O'Meara 2016).

Based on our hypothesis, we created 13 models to test whether and how gamergate affects the diversification of Ponerinae (Tables S2 and S4). In three models, the hidden state is not considered, presenting variation in trait related speciation and extinction rates. The other 10 models were created considering the hidden states to state 1 (gamergate). Such models are: 1) take into account that the evolution of Ponerinae was drive by variation of both speciation and extinction along the three states; 2) consider that gamergate states (1A and 1B) present the same diversification rate, but differs from non-gamergate species (0A); 3) consider that the three states present the same diversification pattern; 4) consider that the three states presents different diversification pattern, driven by speciation, and; 5) consider that the three states present different diversification pattern, driven by extinction. To each of five models we consider two distinct transition scenario (generating ten hidden states models), the first we allow a free transition parameters along the three states. The second, we built an irreversible transition scenario, where gamergate species (1A) are unable to regress to non-gamergate state (0A), as predict by Takebayashi and Morrell (2001) to dead end scenarios. All models were fitted to each of the crown and stem phylogenetic trees.

We used the Akaike Information Criterion (AIC) and model weight to choose among models, with the lower AIC value and highest weight, the corresponding best-fitted model. Then, for each of the 13 models, we calculated the mean AIC value and model weight for all one hundred crown and stem phylogenies, yielding 13 average values. HiSSE analysis was performed in *R* software version 4.2.2 (R Core Team 2022) using the *hisse* package (Beaulieu and O'Meara 2016).

Results

We did not find reliable evidence of negative diversification in Ponerinae species with gamergate trait (Table 1 and Figure S1 and S2). In addition, models that present irreversible transition from gamergate to non-gamergate presented bad fit, contrary to a dead-end scenario (Figure 2 and Table S2 and S3). Models that ignored a hidden state also showed a bad fit. Such a result may indicate that the species with gamergate probably present the influence of others unmeasured traits on diversification rate.

However, we are able to test the two main assumption of a dead-end scenario (negative diversification and irreversible transition), the phylogenetic uncertainty impedes a more detailed description of diversification scenario of Ponerinae. The best-fitted models, summarized in Table 1, present different assumptions about diversification scenarios (Table S2 and S3).

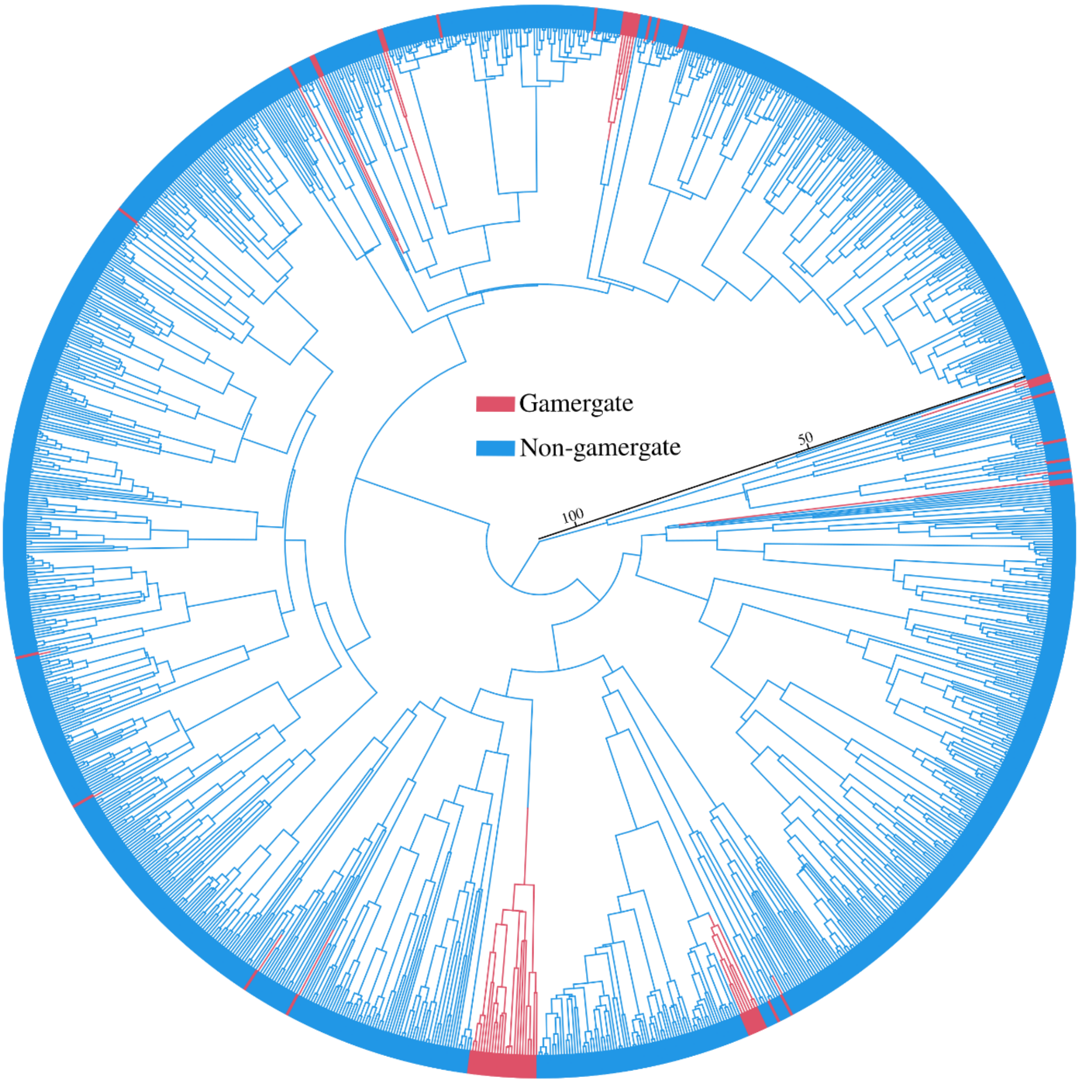

Figure 1. One sampled tree, from Economo et al (2018), shows the distribution of gamergate (red branches) and non-gamergate lineages (blue branches) along Ponerinae tree. Although few species present the trait, gamergate evolved independently several times along Ponerinae evolution.

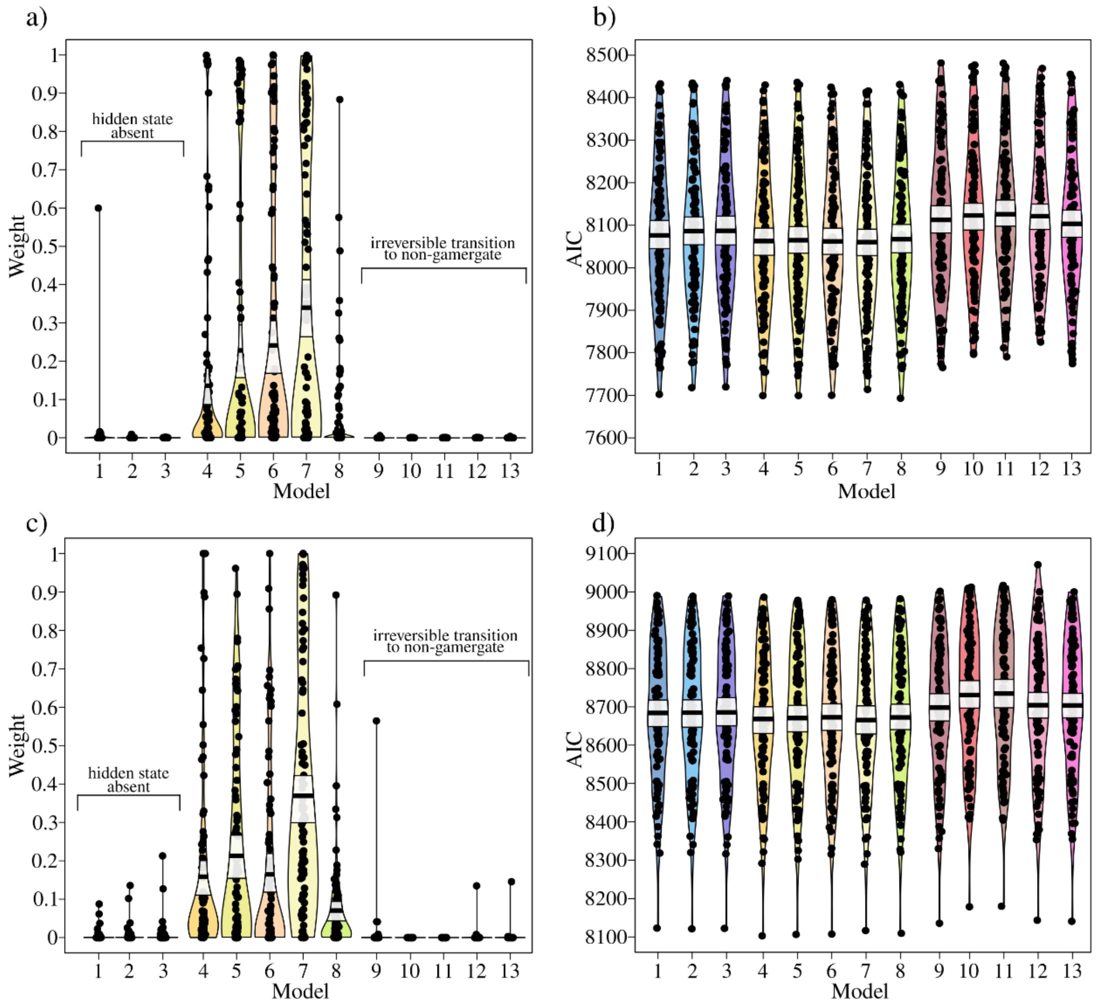

Figure 2. Distribution of model AIC and model weight to all models used here. In general, models that not consider a hidden state and models that consider a hidden state with irreversible transition from gamergate to non-gamergate presented bad fit. Model weight (a) and AIC values (b) to crown trees and model weight (c) and AIC values (d) to stem trees.

Table 1. Summary of best-fitted hisse models. λ= speciation rate, μ= extinction rate, *q*= transition rate. *0A* represents a state where the gamergate trait is absent. *1A*, represent state where gamergaete is present disregarding a hidden state, and in *1B* represents presents a gamergate state considering a hidden state.

Model	**λ0A**	**λ1A**	**λ1B**	**μ0A**	**μ1A**	**μ1B**	**q 0A→1A**	**q 1A→0A**	**q 1A→1B**	**q 1B→1A**
Stem trees										
M. 7	0.105	0.13	0.118	0.043	0.06	0.046	0.039	0.048	0.026	0.001
M. 5	0.106	0.094	0.094	0.043	0.052	0.052	0.041	0.094	0.02	0.006
M. 6	0.106	0.106	0.106	0.048	0.048	0.048	0.072	0.152	0.044	0.001
M. 4	0.106	0.082	0.098	0.044	0.02	0.035	0.068	0.11	0.037	0.002
Crown trees										
M. 7	0.154	0.112	0.35	0.09	0.069	0.205	0.019	0.052	0.028	0.025
M. 6	0.156	0.156	0.156	0.095	0.095	0.095	0.099	0.26	0.045	0.004
M. 5	0.154	0.254	0.254	0.09	0.218	0.218	0.03	0.168	0.071	0.073
M. 4	0.154	0.098	0.4	0.088	0.158	0.234	0.01	0.04	0.019	0.083

Discussion

We present an assessment of the macroevolutionary trends triggered by a form of worker reproductive protagonism in ants - the gamergate. Although the queen is the most important reproductive caste in ants, the long-term effect of reproduction by fertile workers is not negligible for ant evolution. The presence gamergate in ant species was usually associated with positive diversification rates, and present a reversible transition to non-gamergate state: The hypothesis that the gamergate trait leads to an evolutionary dead end was not supported. Furthermore, the rejection of HiSSE models without hidden state suggests that the gamergate attribute itself does not act as the major driver of Ponerinae diversification, with one or more unmeasured traits also playing a role in evolution of such ants.

In fact, some genera as *Diacamma* (44 species), *Dinoponera* (8 species) and *Streblognathos* (2 species) present reproduction exclusively by workers (Peeters and Ito 2001; Bolton, 2023), which could indicate that, once established, the gamergate would not revert to a queen reproduction. However, our results suggest that such a trait is lost in some lineages along time, with the ant queen retake the reproduction of colony. In addition, the best-fitted models also indicate a higher transition rate for loss of the gamergate trait than the opposite, suggesting that the trait may be evolutionarily costly on longer evolutionary timescales. Such results indicate that although does not lead to an evolutionary dead end, the gamergate trait may be disadvantageous in a certain degree.

Although the HiSSE method might overestimate transition rates when a state has low frequency (Beaulieu and O'Meara 2016), biological features of fertile workers could also explain such unbalanced transition rate. Workers present smaller fertility than ant queen (Peeters et al. 2000), which lead to smaller colonies than queenright species (Monnin and Peeters 2008). Colony size is positively related with colony survival (Kramer et al. 2014; Cole et al. 2022), colony defense (Leclerc and Detrain 2018) and diversification (Blanchard and Moreau 2016). In addition, Liebig and Poethke (2004) demonstrate that, for some reason, colonies with reproduction by fertile workers present a mortality rate four times high than queenright colonies. Although fertile workers present longer lifespans than unfertilized workers, such workers show a lesser lifespan than a queen (Schneider et al. 2011). Taken together, the emergence of fertile workers may be advantageous in short timescales, once preventing the death of the colony when the queen dies, for example. However, the same eco-evolutionary features listed above, could explain why, at long timescales, we found an unbalanced transition of reproduction mode in Ponerinae ants.

Due to phylogenetic uncertainty, the diversification scenario of gamergate and non-gamergate lineages remain relatively obscure. In general, the rejection of models that disregard a hidden state (Table S2 and S3) indicate that others unmeasured traits also play role in diversification of lineages with gamergate trait. In addition, the best-fitted models usually present a positive diversification rate to gamergate species (Table 1, Figure S1 and S2) but, as such models present distinct evolutionary predictions, a description of more detailed diversification patterns is unreliable. However, the absence of negative diversification to gamergate species unable us to test if such trait lead to an evolutionary dead end (Takebayashi and Morrell 2001), despite of phylogenetic uncertainty.

The emergence of fertile egg-laying workers is rare in ants, occurring in about 1% of ant species, but more common in the Ponerinae, in about 6% of species, having evolved independently several times. The factors leading to the emergence of fertile workers have been elucidated from morphological (Peeters and Fisher 2016), behavioral (Baudry et al. 2003), and neuronal (Gospocic et al. 2017) perspectives. Here, we added a macroevolutionary perspective to the consequences of fertile workers in ant evolution. Based on currently knowing of gamergate, we found no evidence that the reproduction by workers in caste systems lead to an evolutionary dead end to such species. In terms of future perspectives, a broader knowledge on genetic diversity in gamergate and non-gamergate species as well more detailed descriptions on dispersal modes across Ponerinae species might help answer the relative importance of each life trait for the diversification of such ant group.

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Supplementary material

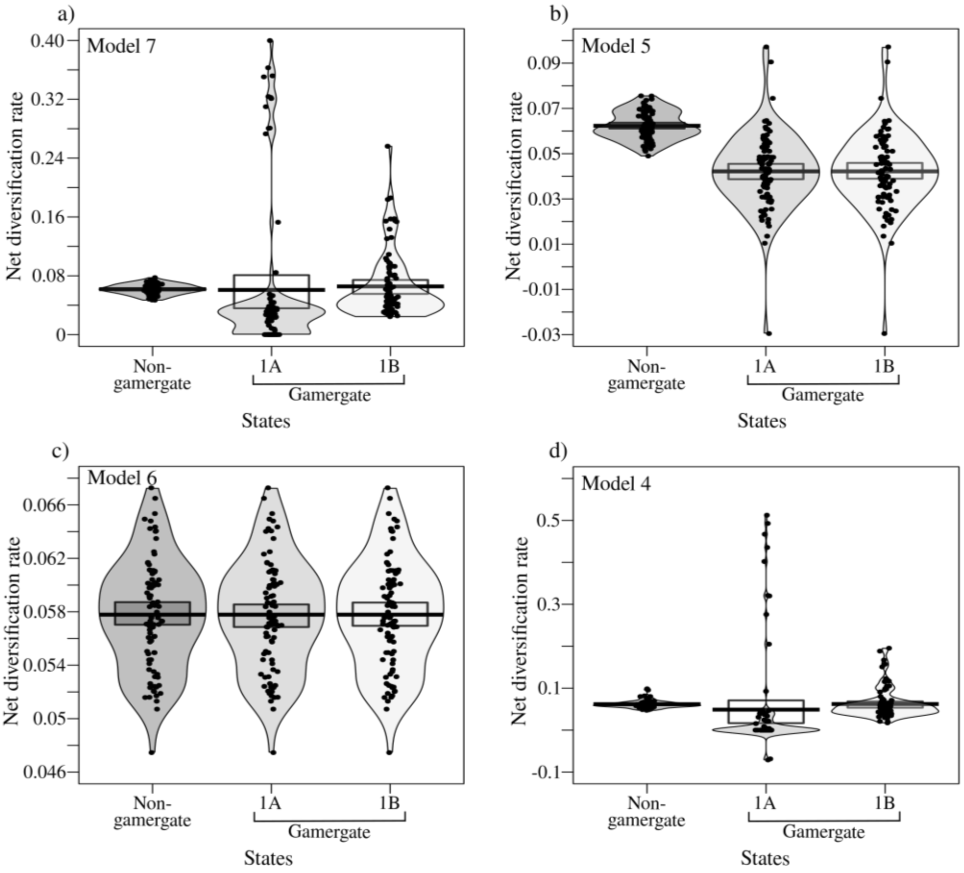

Figure S1. Net diversification rate based on models 4-7, to stem trees. State 1 represent species with gamergate trait, disregarding (1A) or not (1B) a hidden state. Each dot represent the mean diversification rate to each tree.

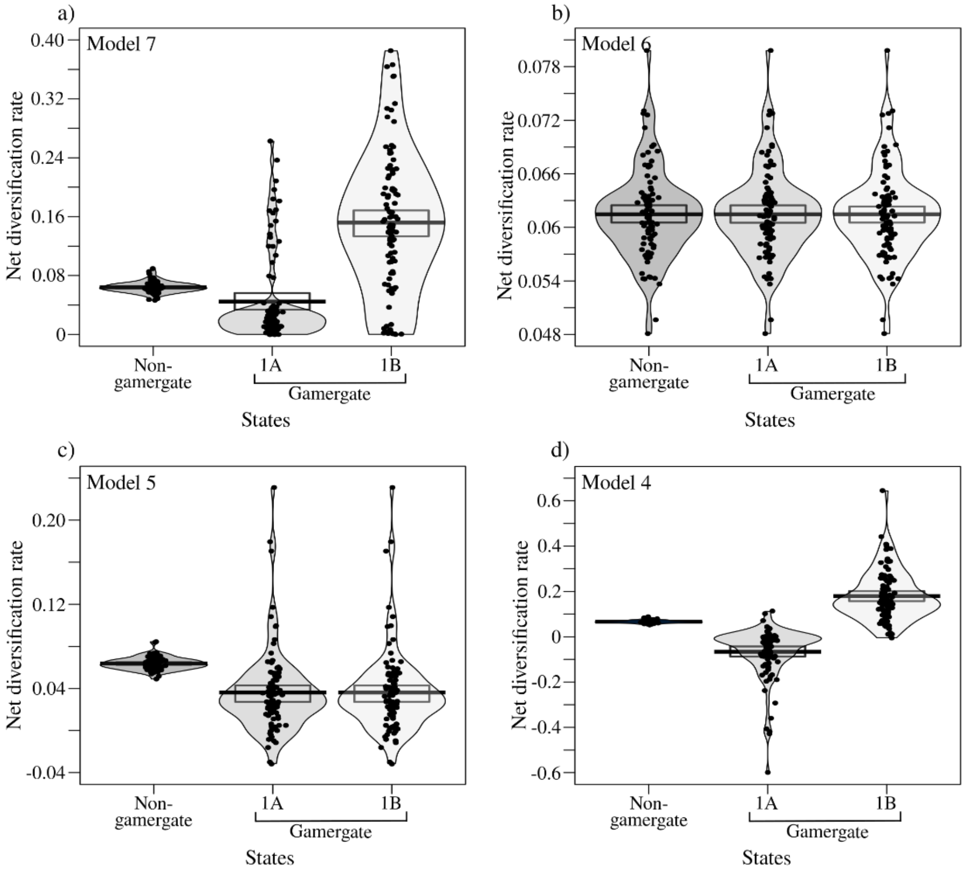

Figure S2. Net diversification rate based on models 4-7, to crown trees. State 1 represent species with gamergate trait, disregarding (1A) or not (1B) a hidden state. Each dot represent the mean diversification rate to each tree.

Table S1. List of 65 Ponerinae species with gamergate workers.

Species	**Reference**
Brachyponera nigrita	Peeters, 1993
Bothroponera kruegeri	Peeters,1991
Bothroponera tesseronoda	Ito, 2010
Diacamma assamense	AntWeb, 2022
Diacamma australe	Peeters,2012
Diacamma baguiense	AntWeb, 2022
Diacamma bispinosum	AntWeb, 2022
Diacamma ceylonense	Monnin and Peeters, 2008
Diacamma colosseense	AntWeb, 2022
Diacamma cupreum	AntWeb, 2022
Diacamma cyaneiventre	André et al., 2006
Diacamma holosericum	AntWeb, 2022

Diacamma indicum	Viginier et al., 2004
Diacamma intricatum	AntWeb, 2022
Diacamma jacobsoni	AntWeb, 2022
Diacamma leve	AntWeb, 2022
Diacamma longitudinale	AntWeb, 2022
Diacamma palawanicum	AntWeb, 2022
Diacamma pallidum	AntWeb, 2022
Diacamma panayense	AntWeb, 2022
Diacamma purpureum	AntWeb, 2022
Diacamma rugivertex	AntWeb, 2022
Diacamma rugosum	Peeters,1991
Diacamma scalpratum	Peeters et al., 2015
Diacamma concentricum	AntWeb, 2022
Diacamma geometricum	AntWeb, 2022
Diacamma schoedli	AntWeb, 2022
Dinoponera grandis	Peeters,1991
Dinoponera quadriceps	Peeters,1991
Dinoponera hispida	Peeters,1991
Dinoponera longipes	Peeters,1991
Dinoponera lucida	Peeters,1991
Dinoponera mutica	Peeters,1991
Dinoponera gigantea	Peeters,1991
Ectomomyrmex leeuwenhoeki	Ito et al., 2007
Ectomomyrmex astutus	Gobin et al., 2008
Euponera sikorae	Peeters and Fisher, 2016
Hagensia havilandi	Villet,1992
Hagensia peringueyi	Schmidt & Shattuck, 2014
Harpegnathos saltator	Peeters et al., 2000
Harpegnathos venator	Aupanun et al., 2022
Leptogenys peuqueti	Peeters,2012
Leptogenys schwabi	Peeters,1991
Leptogenys unistimulosa	Lattke, 2011
Ophthalmopone berthoudi	Peeters,1991
Ophthalmopone depilis	Schmidt & Shattuck, 2014
Ophthalmopone hottentota	Peeters,1991
Ophthalmopone ilgii	Schmidt & Shattuck, 2014
Ophthalmopone mocquerysi	Schmidt & Shattuck, 2014
Platythyrea arnoldi	Villet, 1993
Platythyrea cribrinodis	Villet, 1991a
Platythyrea lamellosa	Villet et al., 1990
Platythyrea punctata	Schilder et al., 1999
Platythyrea quadridenta	Ito, 1995
Platythyrea schultzei	Villet, 1991b
Platythyrea tricuspidata	Ito, 1995

Pseudoneoponera insularis	Ito, 1993
Pseudoneoponera porcata	Peeters,1991
Pseudoneoponera sublaevis	Peeters et al., 1991
Pseudoneoponera tridentata	Sommer et al., 1994
Streblognathus aethiopicus	Robertson, 2002
Streblognathus peetersi	Robertson, 2002
Thaumatomyrmex atrox	Delabie and Pezon, 2000
Thaumatomyrmex mutilatus	Jahyny, 2010
Thaumatomyrmex contumax	Delabie and Pezon, 2000

Table S2. Akaike model selection of 13 HiSSE models of stem trees. Models one to three does not present a hidden state. τ (tau) = speciation + extinction rate, ε = extinction fraction (extinction/speciation) and, *q* = transition rate. 0 and 1 after model parameters represent absence or presence of the gamergate trait, respectively. A and B after model parameters mean a not hidden or hidden state, respectively. Models that consider an irreversible transition from gamergate to non-gamergate (q1A→0A irr.) present the other transition as free. Models 1-3 does not present a hidden state (1B). AIC and weight values are represent by the mean and standard deviation of 100 phylogenetic trees. *P* refers to the number of parameters in each model.

Model	**P**	**AIC (mean ± SD)**	**w (mean± SD)**
7. HiSSE, τ’s all free, ε’s all equal, q’s free	8	8665.44±176.69	0.37± 0.317
5. HiSSE, τ1A= τ1B, *ε*0A=*ε*1A, q’s free	8	8670.64±178.5	0.213±0.258
6. HiSSE, τ’s all equal, ε’s all equal, q’s free	6	8672.65±178.5	0.165±0.248
4. HiSSE, τ’s all free, ε’s all free, q’s free	10	8668.15±178.14	0.158±0.236
8. HiSSE, τ’s all equal, ε’s all free, q’s free	8	8672.45±176.85	0.07±0.129
9. HiSSE, τ’s all free, ***ε***’s all free, q1A→0A irr.	9	8697.96±178.18	< 0.01±0.06
3. BiSSE, τ ‘s free, ***ε***’s all equal, q’s free	5	8685.48±177.07	< 0.01±0.026
2. BiSSE, τ’s all equal, ***ε***’s all free, q’s free	5	8684.92±177.5	< 0.01±0.018
1. BiSSE, τ ‘s free, ***ε***’s all free, q’ free	6	8684.19±177.65	< 0.01±0.012
13. HiSSE, τ’s all equal, ***ε***’s all free, q1A→0A irr.	7	8703.55±177.67	< 0.01±0.015
12. HiSSE, τ’s all free, *ε*’s all equal, q1A→0A irr.	7	8704.44±179.7	< 0.01±0.014
10. HiSSE, τ1A= τ1B, *ε*0A=*ε*1A, q1A→0A irr.	7	8730.65±175.57	< 0.001±< 0.001
11. HiSSE, τ’s all equal, *ε*’s all equal, q1A→0A irr.	5	8734.84±174.9	< 0.001±< 0.001

Table S3. Akaike model selection of 13 HiSSE models of crown trees. Models one to three does not present a hidden state. τ (tau) = speciation + extinction rate, ε = extinction fraction (extinction/speciation) and, *q* = transition rate. 0 and 1 after model parameters represent absence or presence of the gamergate trait, respectively. A and B after model parameters mean a not hidden or hidden state, respectively. Models that consider an irreversible transition from gamergate to non-gamergate (q1A→0A irr.) present the other transition as free. Models 1-3 does not present a hidden state (1B). AIC and weight values are represent by the mean and standard deviation of 100 phylogenetic trees. *P* refers to the number of parameters in each model.

Model	**P**	**AIC (mean)**	**w (mean)**
7. HiSSE, τ’s all free, ε’s all equal, q’s free	8	8060.08±168.57	0.34±0.377
6. HiSSE, τ’s all equal, ε’s all equal, q’s free	6	8061.98±169.13	0.24±0.339
5. HiSSE, τ1A= τ1B, *ε*0A=*ε*1A, q’s free	8	8064.53±170.33	0.23±0.36
4. HiSSE, τ’s all free, ε’s all free, q’s free	10	8062.51±168.37	0.136±0.259
8. HiSSE, τ’s all equal, ε’s all free, q’s free	8	8066.94±169.21	0.048±0.129
1. HiSSE, τ ‘s free, ***ε***’s all free, q’ free	6	8075.95±170.01	< 0.01±0.06
2. HiSSE, τ’s all equal, ***ε***’s all free, q’s free	5	8086.3±167.9	< 0.001±<0.001
9. HiSSE, τ’s all free, ***ε***’s all free, q1A→0A irr.	9	8112.3±173.4	< 0.001±<0.001
13. HiSSE, τ’s all equal, ***ε***’s all free, q1A→0A irr.	7	8103.07±167.43	< 0.001±<0.001
3. HiSSE, τ ‘s free, ***ε***’s all equal, q’s free	5	8086.76±169.03	< 0.001±<0.001
12. HiSSE, τ’s all free, *ε*’s all equal, q1A→0A irr.	7	8121.02±160.5	< 0.001±<0.001
11. HiSSE, τ’s all equal, *ε*’s all equal, q1A→0A irr.	5	8125.68±166.12	< 0.001±<0.001

10. HiSSE, $\tau 1A= \tau 1B$, $\varepsilon 0A=\varepsilon 1A$, q1A→0A irr.	7	8122.93±167.05	< 0.001±<0.001

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CONCLUSÕES

O primeiro capítulo da tese descreve como se deu a evolução espacial das formigas Ponerinae. A partir do seu centro de origem, embora ainda não totalmente definido dado a incerteza filogenética, mas sugerido como sendo o continente Americano, Ponerinae se espalhou pelo pantrópico nos últimos 100 milhões de anos. Atualmente Ponerinae obedece um claro gradiente latitudinal de riqueza. Contudo, tal gradiente não se deve a diferenças nas taxas de diversificação em relação à latitude. Isso indica que tal padrão latitudinal pode ser explicado pela maior idade das linhagens nas regiões tropicais (Economo et al., 2018), e por conseguinte, maior tempo em diversificação.

Ao mesmo tempo, a divisão da distribuição das formigas em biorregiões em uma maneira muito similar a obtida recentemente para vertebrados (Holt, 2013) indica que o isolamento entre essas regiões, em certo grau, gera padrões semelhantes de distribuição de linhagens de vertebrados e insetos. Indo além, a riqueza de espécies em cada biorregião não é definida pela taxa média de diversificação em cada biorregões, mas sim pela área de cada biorregião. Contudo, biorregiões formadas por ilhas ou arquipélagos apresentam uma taxa de diversificação maior que biorregiões continentais. Isso aliado ao fato de gêneros com maior riqueza apresentarem maior área de distribuição indica que dispersão transoceânica, e, em suma, a alopatria exerce um efeito positivo sob a riqueza de Ponerinae.

O capítulo II demonstra que a diversificação temporal de Ponerinae e Myrmicinae não ocorreu como radiações evolutivas sequenciais (primeiro Ponerinae, seguido de Myrmicinae), conforme sugerido por Wilson & Hölldobler (2005). Apesar da relativa incerteza filogenética, as duas subfamílias parecem ter levemente e constantemente aumentado a diversificação, até próximo ao presente, quando apresentaram maior aumento nas taxas de diversificação. Apesar de serem espécies preferencialmente florestais, não foi encontrada evidência de que a expansão florestal após a extinção do Cretáceo-Paleógeno tenha desencadeado um aumento na diversificação de Ponerinae. Em adição, Myrmicinae também não parece ter rapidamente diversificado ao longo do Mioceno, como Wilson & Hölldobler (2005) sugeriram, mas seguiram um padrão de diversificação semelhante à Ponerinae.

Juntamente com a evolução temporal e espacial, a reprodução por operárias (chamadas gamergate) ao invés da rainha, evoluiu diversas vezes de modo independente em Ponerinae. Tal modo reprodutivo já foi sugerido como sendo menos vantajoso que a reprodução pela rainha, gerando, por exemplo, menor tamanho de colônia e maior mortalidade de colônias que colônias com reprodução via rainha (Liebig & Poethke, 2004; Monnin & Peeters 2008). Contudo, como visto no capítulo III, não foram encontradas evidências que a evolução do comportamento reprodutivo em operárias leve as espécies para um beco sem saída evolutivo. Isso é; 1) espécies que se reproduzem via gamergate não apresentam diversificação negativa ao longo do tempo; 2) as linhagens que apresentam reprodução via gamergate provavelmente perdem tal modo reprodutivo, com a rainha voltando a assumira reprodução da colônia, ao longo da evolução de tais espécies.

A presente tese buscou elucidar com um grupo milimétrico de seres vivos se tornou tão especioso ao longo do tempo e quais as consequências de sua intensa dispersão ao longo de todos os continentes, exceto Antártida. Além de testar uma das principais hipóteses em relação à evolução de formigas, também foi possível descrever como o espaço afetou a evoluão de Ponerinae, evidenciando, inclusive, padrões evolutivos similares, encontrados em outros grupos taxonômicos, como os vertebrados. Sendo organismos eussociais, que implica em divisão de tarefas, inclusive reprodutivas, vimos como a reprodução mediada por mais de uma casta pode influenciar a evolução de organismos sociais ao longo do tempo. Por fim, para além dos estudos conhecidos em plantas e vertebrados, principalmente, a presente tese amplia o horizonte de entendimento sobre como os insetos evoluíram em grandes escalas de tempo e espaço.

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