INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA
PROGRAMA DE PÓS-GRADUAÇÃO ECOLOGIA

Protocolo simples para gravação acústica de formigas (Hymenoptera: Formicidae) e relações entre o comportamento de dominância numérica e características morfológicas nos sinais acústicos de formigas do solo

ÉRICA MARINHO DO VALE

Manaus, Amazonas
Fevereiro 2024

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PROTOCOLO SIMPLES PARA GRAVAÇÃO ACÚSTICA DE FORMIGAS (HYMENOPTERA: FORMICIDAE) E RELAÇÕES ENTRE O COMPORTAMENTO DE DOMINÂNCIA NUMÉRICA E CARACTERÍSTICAS MORFOLÓGICAS NOS SINAIS ACÚSTICOS DE FORMIGAS DO SOLO

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Dissertação apresentada ao Instituto Nacional de Pesquisas na Amazônia como parte dos requisitos para obtenção do título de Mestre em Biologia (Ecologia).

Manaus, Amazonas
Fevereiro 2024

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 28 dias do mês de Fevereiro do ano de 2024, às 09:h00min, via videoconferência, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o Dr. **Pedro Aurélio Costa Lima Pequeno**, da Universidade Federal de Roraima – UFRR, a Drª. **Itanna Oliveira Fernandes**, do Instituto Nacional de Pesquisas da Amazônia – INPA e a Drª. **Mariane Bosholn**, da Universidade Federal de Roraima – UFRR, sendo os suplentes a Drª. Patrícia Ferreira Monticelli, da Universidade de São Paulo – USP e o Dr. Raphael Aquino Heleodoro, do Instituto Nacional de Pesquisas da Amazônia – INPA, sob a presidência do orientador, a fim de proceder a arguição pública da **DISSERTAÇÃO DE MESTRADO** de **ÉRICA MARINHO DO VALLE**, intitulado: **"RELAÇÕES ENTRE OS SINAIS ACÚSTICOS E TRAÇOS MORFOLÓGICOS E COMPORTAMENTAIS DE FORMIGAS DE SOLO EM FLORESTAS DE TERRA FIRME, AMAZÔNIA CENTRAL, MANAUS (AM) "**, orientada pelo Dr. Paulo Estefano Dineli Bobrowiec, do Instituto Tecnológico Vale – ITV e coorientada pelo Dr. Fabricio Beggiato Baccaro, da Universidade Federal do Amazonas – UFAM.

Após a exposição, o(a) discente foi arguido oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

☒ APROVADO (A) ☐ REPROVADO (A)

☒ POR UNANIMIDADE ☐ POR MAIORIA

Nada mais havendo, a presente ata foi lida, lavrada e assinada pelos membros da Comissão Examinadora.

DR. PEDRO AURÉLIO COSTA LIMA PEQUENO	Pedro Aurélio Costa Lima Pequeno
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DR. RAPHAEL AQUINO HELEODORO	

Marina Anciães
(Coordenação PPG-ECO/INPA)

Catalogação na Publicação (CIP-Brasil)

V149p Vale, Érica
Protocolo simples para gravação acústica de formigas (*Hymenoptera: Formicidae*) e relações entre o comportamento de dominância numérica e características morfológicas nos sinais acústicos de formigas do solo / Érica Marinho do Vale; orientador Paulo Estefano Bobrowiec; coorientador Fabrício Beggiato Bacarro,. - Manaus : [s.l.], 2024.

4.086 mb
73p. : il. color.

Dissertação (Mestrado - Programa de Pós-Graduação em Ecologia) - Coordenação do Programa de Pós-Graduação, INPA, 2024.

1. Bioacústica. 2. Protocolo acústico. 3. Dominância númerica. I.Bobrowiec, Paulo Estefano. II. Beggiato Bacarro, Fabrício. III. Título.

CDD 595.796

Sinopse: Nessa dissertação, desenvolvemos um protocolo de gravação de sinais acústicos de espécies de formigas e avaliamos a identificação das espécies com base nos parâmetros espectro-temporais desses sinais. Investigamos também se a emissão desses sinais influencia a dominância por recursos alimentares e a competição entre espécies. Além disso, avaliamos como a dominância numérica e a morfologia do aparato estridulatório afetam os parâmetros espectro-temporais dos sinais acústicos em formigas da Amazônia Central.

Palavras-chave: sinais acústicos, formigas, identificação de espécies, dominância, recrutamento, Amazônia Central

Dedico esta dissertação à minha mãe Ana Telma.

AGRADECIMENTOS

Primeiramente, agradeço a Deus, aos meus guias espirituais e à Mãe Natureza por me concederem força e foco ao longo dessa jornada. Sem essa conexão, a conclusão desta pesquisa não teria sido possível.

Agradeço profundamente à minha família, à minha mãe, Ana Telma, e ao meu pai, Dirne, por me proporcionarem, por meio da educação, as oportunidades que me permitiram alcançar o conhecimento. Sou igualmente grata ao meu irmão, Diogo, pelo constante apoio. Agradeço também aos meus avós, Amélia e Santos, que sempre cultivaram em mim o amor pela natureza, com destaque para meu avô Santos, cuja inspiração foi uma verdadeira motivação ao longo da minha trajetória.

Meus sinceros agradecimentos vão aos meus orientadores desde a graduação, Fabrício Baccaro e Tainara Sobroza, por toda a atenção, ensinamentos, disponibilidade e pelas inúmeras oportunidades oferecidas. Durante o mestrado, agradeço novamente a Fabrício Baccaro, juntamente com Paulo Bobrowiec, pelo apoio incondicional no desenvolvimento desta pesquisa, mesmo diante dos desafios impostos pela pandemia da COVID-19.

Um agradecimento especial ao professor Fabrício Baccaro, não apenas por sua orientação dedicada, mas também por acreditar em mim, mesmo quando eu mesma duvidava. Sua contribuição ao meu crescimento acadêmico e científico é inestimável.

Agradeço ao meu namorado Lorran e à minha sogra Luciane, que sempre estiveram ao meu lado e se dispuseram prontamente a me ajudar nas expedições de campo. Sua companhia e apoio foram fundamentais para o sucesso deste trabalho. Agradeço também ao amigo Jefferson, que, com sua disposição e colaboração, contribuiu imensamente nas atividades de campo.

Agradeço aos companheiros de campo Seu José, Fagner, Pinduca e Juruna, cujo conhecimento e disposição tornaram essa experiência mais enriquecedora. Aos amigos do Laboratório de Ecologia da UFAM, especialmente Cláudio, Thalita, Cláudia, Amanda, Isabella, e muitos outros, sou grata por cada ensinamento, partilha e apoio durante esses anos.

Ao meu fiel companheiro de quatro patas, Lawrence, agradeço por sua parceria e apoio emocional incondicional ao longo deste percurso.

Agradeço à Juliane, secretária do PPG-ECO, por sua assistência sempre gentil e precisa.

Sou grata aos colegas dos Laboratórios **LABECA** e **Plâncton** pelo incentivo constante na pesquisa. Agradeço ao Instituto Nacional de Pesquisas da Amazônia **- INPA**, ao **CMABio** da **UEA**, à **UFAM** e ao **CEBAM** pela infraestrutura, e à **FAPEAM** pelo apoio financeiro essencial ao desenvolvimento deste trabalho. Meu agradecimento a todos que, de alguma forma, contribuíram para a realização desta pesquisa.

RESUMO

As formigas emitem sinais acústicos através de percussão, usando o corpo, ou estridulação, através do aparato estridulatório. Embora os estudos analisem o comportamento de emissão de sinais e utilizem sinais acústicos para identificar espécies de formigas, ainda não sabemos como a emissão desses sinais influencia a dominância por recursos alimentares e, consequentemente, a competição por eles. Nesta dissertação, (i) desenvolvi um protocolo simples e econômico para gravar sinais acústicos de formigas em dois contextos ecológicos: recrutamento para uso de recursos e chamadas de socorro; e (ii) investiguei como a dominância numérica e a morfologia do aparato estridulatório afetam a emissão de sinais acústicos e os parâmetros espectro-temporais das formigas da Amazônia Central. Entre 70 espécies, 38 produziram sinais acústicos em ambos os contextos. No entanto, usamos apenas 28 espécies de 7 subfamílias que foram registradas para análise. Nossos resultados sugerem que, em média, combinações de microfones e gravadores identificaram as espécies corretamente em 55 a 90% das vezes, e o uso de parâmetros temporais aumenta a precisão dos modelos, indicando que a análise de sinais acústicos é mais eficaz com parâmetros espectrais e temporais. Em relação ao comportamento de dominância numérica, os sinais acústicos são mais emitidos por formigas subordinadas, em comparação às formigas dominantes, durante o forrageamento. A altura do aparato estridulatório está correlacionada com o número de pulsos do sinal, mas o intervalo médio entre os pulsos não está relacionado à distância entre as cristas. Por fim, esperamos contribuir para estudos de comportamento e evolução da comunicação acústica dentro do grupo das formigas.

Palavras-chave: sinais acústicos, formigas, Amazônia Central, comportamento, recrutamento

ABSTRACT

Ants emit acoustic signals through percussion, using their bodies, or stridulation, through the stridulatory apparatus. Although studies analyse the behaviour of signal emission and use acoustic signals to identify ant species. It remains unclear how the emission of these signals influences resource dominance and, consequently, competition for food. In this dissertation, (i) I developed a simple and cost-effective protocol to record acoustic signals from ants in two ecological contexts: recruitment for resource use and distress calls; and (ii) I investigated how numerical dominance and the morphology of the stridulatory apparatus affect the emission of acoustic signals and the spectro-temporal parameters of ants in Central Amazonia. Among 70 species, 38 produced acoustic signals in both contexts. However, we used only 28 species from 7 subfamilies that were recorded for analysis. Our results suggest that, on average, combinations of microphones and recorders correctly identified the species 55 to 90% of the time, and the use of temporal parameters increased the precision of the models, indicating that acoustic signal analysis is more effective with spectral and temporal parameters. Regarding numerical dominance behaviour, acoustic signals are more frequently emitted by subordinate ants compared to dominant ants during foraging. The height of the stridulatory apparatus is correlated with the number of signal pulses, but the average interval between pulses is not related to the distance between ridges. Finally, we hope to contribute to studies of behaviour and the evolution of acoustic communication within the ant group.

Keywords: acoustic signals, ants, Central Amazon, behavior, recruitment

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INTRODUÇÃO GERAL

A comunicação das formigas é um fenômeno multimodal, englobando diferentes mecanismos, tais como sinais químicos, táteis e acústicos (Markl 1965; Hölldobler 1999. Em formigas, a forma mais conhecida de comunicação intraespecífica é a emissão de substâncias químicas voláteis, substâncias solúveis e o tato, que juntos formam os feromônios (Hölldobler and Wilson 1990). O feromônio de recrutamento, em particular, é depositado no substrato em forma de trilhas, indicando a presença e a localização de fontes alimentares (Hölldobler and Wilson 1990). Entretanto, evidências sugerem que a emissão de sinais acústicos pode maximizar a propagação de sinais mediados por feromônios (Hölldobler 1978). Durante o comportamento de recrutamento de operárias, as formigas podem emitir sinais acústicos ou estridulatórios para complementar ou modificar as informações transmitidas por sinais químicos (Hölldobler et al. 1978; Baroni Urbani et al. 1988; Roces et al. 1996).

Os sinais acústicos em formigas podem ser produzidos por diferentes comportamentos, como a percussão (*drumming behaviour*), que envolve bater partes do corpo sobre o substrato, e a estridulação (*stridulation behaviour*), que ocorre quando há a presença de um aparato especializado, como projeções de estruturas quitinosas (Hölldobler e Brown 2000). A estridulação é definida como a geração de sons pela fricção de regiões diferenciadas do exoesqueleto (Leston e Pringle 1963). Em formigas, esse movimento de fricção no aparato estridulatório é provocado pelo movimento para cima e para baixo do gáster, gerando sinais acústicos conhecidos como *chirps* (Ewing 1989; Hickling e Brown 2000).

A morfologia do aparato estridulatório envolve duas partes: um raspador (*plectron*) localizado na borda do pós-pecíolo ou no primeiro segmento do gáster, dependendo da espécie, e uma estrutura modificada (*pars stridens*), localizada na extremidade anterior do primeiro tergito gástrico. Essas estruturas possuem cristas cuticulares dispostas em finas camadas paralelas, chamadas de estrias (Hölldobler e Wilson 1990; Yao et al. 2018). Sobre os *pars stridens*, existem relevos tegumentares chamados pilares, que são estruturas de suporte com função amplificadora do som (Álvarez 2009). Além disso, a presença de sacos de ar expandidos dentro do gáster, em contato com a cutícula externa, também auxilia na amplificação do som, como observado em *Solenopsis richteri* (Forel 1909) (Hickling e Brown 2000).

Há evidências da relação entre o tamanho do corpo e o tamanho dos *pars stridens*, onde espécies maiores possuem *pars stridens* maiores. No entanto, o tamanho médio das cristas dos *pars stridens* não depende do tamanho da formiga, como constatado em algumas espécies da subfamília Myrmicinae (Castro et al. 2015). Essa variação morfológica parece influenciar as frequências emitidas, que podem ser compostas por frequências baixas (< 20 kHz) ou altas (> 20 kHz), chegando à faixa do ultrassom. Contudo, poucos estudos investigaram a emissão de sinais acústicos em frequências acima de 20 kHz em formigas (Esperson 1994; Pavan e de Carli 1997).

Originalmente, a estridulação em formigas foi atribuída ao sinal de resgate por soterramento em espécies que vivem no solo (Markl 1973; Ruiz et al. 2006; Hunt e Richard 2013). Porém, essa hipótese não foi sustentada após uma reconstrução filogenética do estado ancestral e o mapeamento da presença do aparato estridulatório. A proporção de formigas que apresentam o aparato estridulatório é maior em formigas primariamente arborícolas e evoluiu de forma independente pelo menos cinco vezes nas subfamílias Pseudomyrmicinae, Myrmicinae, Ecatatominae, Paraponerinae e Ponerinae (Golden e Hill 2016). Desta forma, é importante investigar a emissão de sinais acústicos em outros ambientes e contextos comportamentais.

As formigas são insetos sociais que desenvolvem várias tarefas em cooperação, aumentando a eficiência de forrageamento da colônia (Oster e Wilson 1978). O forrageamento é influenciado por fatores como a disponibilidade de alimento, sua distribuição espacial e temporal, acessibilidade, capacidade de carregamento de cada operária e relações de dominância entre espécies de formigas (Levings e Traniello 1981; Schoener 1971; Baccaro et al. 2012; Viana-Bailez e Endringer 2016). As formigas podem forragear de três formas: 1) individualmente, quando as operárias agem de forma independente; 2) por recrutamento, onde operárias isoladas procuram alimento e chamam outras para a coleta; e 3) de maneira especializada, em que operárias caçam em grupo ou parasitam colônias de outras espécies (Oster e Wilson 1978).

O forrageio, por meio do recrutamento e da emissão de sinais acústicos, foi observado principalmente em formigas dos gêneros *Atta* (Fabricius 1804), que cultivam fungos, e *Aphaenogaster* (Mayr 1853). Esse comportamento ocorre especialmente durante o recrutamento de indivíduos, corte de folhas, migração de ninho, encontros agonísticos e cuidado parental das pupas (Mark 1965; Mark 1967; Baroni-Urbani et al. 1988; Roces e Hölldobler 1996; Rauth e Vison 2006). Além disso, algumas espécies de formigas têm a capacidade de receber sinais acústicos e responder a outros insetos mutualistas ou parasitas (Barbero et al. 2009; Cassaci et al. 2014; Giulio et al. 2015; Schönrogge et al. 2016; Lin et al. 2019; Fattorini e Giulio 2021).

A competição e a dominância comportamental são temas amplamente discutidos na ecologia das formigas (Holldobler e Wilson 1990; Cerdá et al. 2013). A dominância comportamental reflete a capacidade de luta, a habilidade de recrutamento de indivíduos e monopolização do alimento (Schoener 1983). Entre as formigas que recrutam, existem pelo menos três níveis hierárquicos de competição (Vespsäläinen e Pisonski 1982): 1) as dominantes, que são espécies agressivas que realizam recrutamento em massa; 2) espécies dominantes ocasionais ou subdominantes, que só conseguem dominar os recursos na ausência de espécies dominantes e 3) as subordinadas, espécies não agressivas que defendem apenas o ninho. Embora diversos estudos tenham demonstrado as relações de dominância hierárquicas em formigas (Vepsäläinen e Pisarski 1982; Fellers 1987; Savolainen e Vepsäläinen, 1988; Andersen 1992, 1997; Sanders et al. 2007; Baccaro e Souza 2007), ainda não está claro como esses padrões de dominância por recurso alimentar influenciam a emissão dos sinais acústicos.

A recepção dos sinais acústicos em formigas ainda é pouco compreendida. Por muito tempo, considerou-se que as formigas recebiam sinais acústicos transmitidos apenas pelo substrato, através de estruturas localizadas nas tíbias, chamadas de órgãos de cordão (ou cordotonais), estrutura já identificada em outras ordens de insetos (Markl 1965; Field e Matheson 1998; Roces e Tautz 2001; Devetak e Delakorda 2004). Porém, é provável que a recepção do sinal acústico também ocorra pelo ar, através de sensilas tricoides especializadas (Dumpert 1972). Essas sensilas estão localizadas principalmente nos segmentos apicais das antenas das formigas (Hashimoto 1990; Hickling e Brown 2000; Renthal et al. 2003). As sensilas são projeções cuticulares responsáveis pela recepção de estímulos que, dependendo de sua estrutura morfológica, podem desempenhar funções receptoras de hidrocarbonetos cuticulares ou gustativas (sensilas basiconicas), hidroreceptores, termorreceptores e mecanorreceptores (sensilas coelocapitulares e sensilas tricoides), além de quimiorreceptores (sensilas tricoides curvadas e sensilas ampuláceas) (Barsagade et al. 2013; Dumpert 1972; Kleineidam et al. 2000; Renthal et al. 2003; Ozaki et al. 2005).

Embora seja conhecido que os aparatos morfológicos estão envolvidos no sistema de produção e na possível recepção de sinais acústicos em formigas, ainda faltam estudos sobre como os parâmetros espectrotemporais desses sinais são influenciados pelas características morfológicas das formigas que estridulam. Além disso, a maioria dos estudos que relacionam a emissão de sinais acústicos com comportamento e morfologia está concentrada em regiões temperadas e subtropicais (Hölldobler 1998; Hickling e Brown 2000; Jackson e Ratnieks 2006; Ferreira et al. 2010; Mankin e Benshemesh 2006; Pielström e Roces 2001; Masoni 2021).

Esta dissertação está organizada em dois capítulos. No capítulo **I**, desenvolvemos e testamos diferentes equipamentos e protocolos de gravação de sinais acústicos emitidos por formigas em dois contextos ecológicos: recrutamento (recruitment behaviour) para fontes de recurso e sinal de socorro (distress call behaviour). No capítulo **II**, investigamos as relações entre o comportamento de dominância numérica e características morfológicas que influenciam a emissão e os parâmetros espectro-temporais dos sinais acústicos em espécies de formigas do solo na Amazônia Central.

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OBJETIVO GERAL

Desenvolver um protocolo simples e barato para gravação de formigas e testar se é possível identificar espécies através desses sinais acústicos, e investigar como o comportamento de dominância numérica e as características morfológicas influenciam a emissão e os parâmetros espectro-temporais de sinais acústicos em espécies de formigas de solo na Amazônia Central.

OBJETIVOS ESPECIFÍCOS

Capítulo I:

Objetivo específico 1.0 Desenvolver um protocolo acessível e eficiente para a coleta e gravação de sinais acústicos de formigas em diferentes contextos ecológicos.

Objetivo específico 1.1 Estimar a acurácia na identificação de espécies de formigas com base nos parâmetros espectro-temporais dos sinais acústicos, considerando os contextos de recrutamento para fontes de alimento e *distress calls*.

Capítulo II:

Objetivo específico 2.1 Investigar a relação entre dominância numérica e a emissão de sinais acústicos por espécies de formigas que recrutam durante forrageio.

Objetivo específico 2.2 Analisar como as características morfológicas influenciam os padrões espectro-temporais dos sinais acústicos emitidos por espécies de formigas do solo na Amazônia Central.

Capítulo 1

VALE E, SHONBERG L, SOBROZA T, BACARRO F (2024**) Establishing a simple acoustic protocol to delimit ants' acoustic temporal-spectral parameters in the Central Amazon**

Estabelecendo um protocolo acústico simples para delimitar os parâmetros espectro-temporais dos sinais acústicos das formigas na Amazônia Central

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RESUMO

Em insetos sociais como as formigas, os sinais acústicos desempenham um papel crucial na facilitação da comunicação rápida em curtas distâncias. Apesar de sua importância, nossa compreensão desses sinais em várias espécies de formigas e comportamentos ainda é limitada. Este estudo apresenta uma metodologia simples e econômica para capturar sinais acústicos em formigas. Investigamos a viabilidade de distinguir espécies de formigas analisando os parâmetros de frequência e temporais de chamadas de recrutamento e de angústia. As gravações foram realizadas em áreas fragmentadas e contínuas da floresta amazônica brasileira, empregando quatro combinações de três microfones e dois gravadores para avaliar o desempenho em diferentes contextos comportamentais, incluindo recrutamento para fontes de alimento e respostas de angústia. Entre as 70 espécies de formigas gravadas, 38 produziram sinais acústicos em ambos os contextos; no entanto, apenas 28 espécies de sete subfamílias foram analisadas sistematicamente. Em média, as combinações de microfones e gravadores identificaram corretamente as espécies com base em seus sinais acústicos entre 55% e 90% das vezes. A incorporação de parâmetros temporais aumentou significativamente a precisão do reconhecimento das espécies, indicando que as análises são mais eficazes quando tanto métricas espectrais quanto temporais são utilizadas. Confiar apenas em parâmetros espectrais resultou em uma redução da precisão da identificação. Este trabalho oferece uma abordagem robusta e acessível para estudar os sinais acústicos de formigas em uma variedade de contextos comportamentais, abrindo caminho para pesquisas futuras sobre a evolução da comunicação acústica em populações de formigas.

Palavras-chave: protocolo, floresta tropical, estridulação, percussão, comportamento de formigas

Establishing a simple acoustic protocol to delimit ants' acoustic temporal-spectral parameters in the Central Amazon

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ABSTRACT

In social insects such as ants, acoustic signals play a crucial role in facilitating rapid communication over short distances. Despite their importance, our understanding of these signals in various ant species and behaviours is still limited. This study presents a simple and economical methodology for capturing acoustic signals in ants. We investigated the feasibility of distinguishing ant species by analysing the frequency and temporal parameters of recruitment and distress calls. Recordings were made in fragmented and continuous areas of rainforest in the Brazilian Amazon, employing four combinations of three microphones and two recorders to assess performance in different behavioural contexts, including recruitment to food sources and distress responses. Among the 70 ant species recorded, 38 produced acoustic signals in both contexts; however, only 28 species from seven subfamilies were systematically analysed. On average, the combinations of microphones and recorders correctly identified the species based on their acoustic signals 55 to 90 per cent of the time. Incorporating temporal parameters significantly increased the accuracy of species recognition, indicating that analyses are more effective when both spectral and temporal metrics are used. Relying on spectral parameters alone resulted in reduced identification accuracy. This work offers a robust and accessible approach to studying the acoustic signals of ants in a variety of behavioural contexts, paving the way for future research into the evolution of acoustic communication in ant populations.

Keywords: protocol, rain forest, stridulation, percussion, ant behavioural

INTRODUCTION

Among ants, chemically mediated signals are considered the primary communication system, although many ant species use other signalling mechanisms, such as the emission of acoustic signals (Hickling and Brown 2000; Jackson and Ratnieks 2006; Baroni-Urbani 1988). Ants use acoustic signals in different behavioural contexts, such as emigration, recruitment, excavation, construction of nest mounds, leaf cutting, parental care, defensive behaviour and alarm (Markl 1965; Mark 1977; Baroni-Urbani et al. 1988; Roces and Hölldobler 1996; Hölldobler 1999; Rauth and Vinson 2006; Sala et al. 2014; Ferreira et al. 2014; Masoni et al. 2021; Roces 2022). The type of acoustic signal may be caste-specific, and acoustic emissions from the sclerotized pupal stage can signal information about an individual's social status in the colony (Barbero et al. 2009; Casacci et al. 2013). More complex mimicry of ant acoustic signals also exists among some beetles, caterpillars, and moth larvae and in some parasitic ant species that occupy other ants' nests (Barbero et al. 2009; Barbero et al. 2012; Schönrogge et al. 2017; Lin et al. 2019; Fattorini 2021, Casacci et al. 2021). These interactions are evidence of the importance of acoustic signals for communication over short distances for various ant species.

Ants can produce acoustic signals through percussion or stridulation. During percussion, ants hit a part of the body directly on a substrate, while stridulation involves rubbing specialized body structures against each other during quick vertical movement of the gaster (Hölldobler and Wilson 1990). These specialized structures are called the stridulatory organ (SO), consisting of a cuticular scraper on the post-petiole (plectron) that rubs against a series of horizontal ridges (*pars stridens*) on the anterior of the first tergite of the gaster (Hölldobler and Wilson 1990; Yao et al. 2018). In the Myrmicinae, the SO is located between the edge of the postpetiole and the gaster. In the Ponerinae, it is located between the first and second segments of the gaster (Ferreira et al. 2010). The alternating movement of the SO can produce sequences of acoustic signals with pulses composed of two energy subunits called disyllabic chirps (Pavan et al. 1997). The SO is typically present in arboreal ant genera and seems to have evolved independently at least five times among the subfamilies Pseudomyrmicinae, Myrmicinae, Ectatomminae, Paraponerinae and Ponerinae (Golden and Hill 2016).

Generally, low-frequency acoustic signals transmitted by the body through the substrate (substrate-borne vibration) can be received by the chordotonal organ (Markl

1965; Roces and Tautz 2001) and possibly by trichoid sensilla present in the antennae (Hashimoto 1990; Hickling and Brown 2000; Renthal et al. 2003). In some species of the Myrmicinae subfamily, acoustic signals are composed mainly of low frequencies, which may increase signal propagation during recruitment (Hickling and Brown 2000). In species of the Ponerinae subfamily, on the contrary, there are no signals of recruitment, but distress calls are emitted in ultrasonic frequencies above 75 kHz (Peña Carrillo et al. 2022). However, there are few investigations on acoustic communication in neither ultrasonic nor low frequencies (bass) in other behavioural contexts and species (Esperson 1994; Pavan et al. 1997).

The use of acoustics is a promising tool in integrative biology studies, which may help to clarify relationships between species (Grasso et al. 2000, Ferreira et al. 2010, Ferreira et al. 2014; Peña Carrillo at al. 2022). The SO morphology, the presence/absence of acoustic signals, and the characteristics of these signals are being used as additional tools in species delimitation. In these studies, most recordings have been made in contexts of the “distress call” (Pavan et al. 1997; Ferreira et al. 2010; Castro et al. 2015; Peña Carrillo at al. 2022). Distress calls are often recorded while handling animals with forceps. This method might be helpful for taxonomic purposes. For instance, it is possible to discern between morphologically cryptic species in the *Pachycondyla apicalis* and *Ectatomma ruidum* complexes (Ferreira et al. 2010; Peña Carrillo et al. 2022). However, a plethora of acoustic signals are emitted by ants in different behavioural contexts (Masoni et al. 2021; Casacci et al. 2021; Peña Carrillo at al. 2022), and as far as we know, there is no simple, cheap and non-invasive protocol to record ant acoustics in their different behavioural contexts.

Recording ants in natural systems and in the laboratory was possible with the development of new acoustic capture tools (Byrne et al. 2022; Peña Carrillo et al. 2022). Accelerometers, laser-doppler-vibrometer, geophones, condenser microphones and ultrasonic microphones are among the recent methods used to record acoustic signals in ants (Pielström and Roces 2012; Carlos et al. 2018; Hickling and Brown 2000, Peña Carrillo et al. 2022). However, there is no simple protocol to record the acoustic communication in typical behavioural interactions of ants, and most of this equipment is expensive or difficult to handle in the field. In addition, most of the information on the characteristics and function of acoustic signals of ants is concentrated in a few species from temperate and subtropical regions focused on communication between castes or distress calls (Hölldobler, 1999; Hickling and Brown, 2000; Jackson and Ratnieks 2006; Ferreira et al. 2010; Mankin and Benshemesh, 2006; Pielström and Roces 2012, Golden and Hill 2016).

Here, we propose a simple protocol based on a contact microphone and an ultrasonic microphone that can help collect acoustic signals emitted by ants at the assemblage level, assisting in studies of the evolution of acoustic signals in other behavioural contexts of terrestrial and arboreal ants. Specifically, (1) we compared the performance of four combinations of three microphones and two low-cost recorders in two contrasting behavioural contexts: recruitment for food sources and distress calls. Using 28 Amazonian species as a model, we used frequency and temporal spectral parameters to test whether it is possible to identify species through their acoustic signals. We also addressed which combination of equipment is more efficient for such a purpose. Finally, (2) we tested the contribution of using temporal parameters, which are more challenging and time-consuming to estimate, in delimiting ant species based solely on acoustic signals.

MATERIAL AND METHODS

Study area and field data

The collections were carried out at the Campus of the Federal University of Amazonas (UFAM) (3º06’01.2’ ’S 59º58’25.5’’W), Experimental Farm of the UFAM (2º38’20’ ’S 2º39’10’ ’S 60º40’W 60º30’W) and Adolpho Ducke Forest Reserve (RFAD) (2°57’42 “S 59°55’40” W), Manaus, Amazonas, Brazil. The collections were carried out during the last five years since the first tentative sampling protocol, between 2017-2023.

We sampled and recorded ants on the main trails of the UFAM campus, an urban forest fragment of about 776 hectares. Campus UFAM is a forest fragment impacted by urban growth (Borges and Guilherme 2013, Marcon et al. 2012). We also collected on the main access trail to the Biodiversity Research Program (PPBIO) grids at the UFAM Experimental Farm, located at km 38 of the BR-174 highway. The Experimental Farm has a pristine terra-firme forest of 3,000 hectares, situated in the rural area of the city of Manaus (Borges and Guilherme 2013). We also collected on the main access trails to the PPBIO grids in Reserva Ducke, located in the peripheral area of Manaus (Baccaro et al. 2008). Most parts of the Ducke reserve are still undisturbed, but some areas closer to the city border already had signs of forest disturbance.

The average annual temperature in the region is 26º C, with slight thermal variation during the year (Baccaro et al. 2008). The climate of the region is classified as humid tropical. There are two distinct seasons: the rainy season between December and May, with March and April having the highest precipitation, and the dry season between June and November, with September being the driest month (Ribeiro and Villa Nova 1979; Baccaro et al. 2008).

Acoustic data collection

The recorded acoustic signals were observed in two behavioural contexts: signals of recruitment by workers during bait recruitment and signals of “stress” or a “distress call.” Recruitment signals were recorded in the forest, with contact microphones attached below the bait (usually below a dry leaf). The bait consisted of 50% maniva flour and 50% canned sardines. We recorded when the first ant arrived at the bait and continued for at least 5 minutes. Distress call signals were recorded by gently gripping the ant with forceps by the mesosoma. Distress call recordings were made in the laboratory, positioning the ultrasonic microphone 5 mm away from the ant or over the contact microphone and immobilizing the ant with forceps. After the field recordings, some workers present at baits and solitary foragers (who do not recruit) were collected to be recorded again at the UFAM Community Ecology Laboratory. We also recorded acoustic warning signals from plant-associated ants engaging the contact microphone in domatia. After recording in the laboratory, the ants were fixed in 95% alcohol for identification (Baccaro et al. 2015).

Equipment

We recorded ant signals in uncompressed wav digital format with a ZOOM HN14 (16-bit resolution, sample rate of 44.0 kHz) and a Sound Devices Mix-Pre 3 II recorder (32-bit resolution, sample rate of 192.0 kHz), combined with one of three microphones: 1) Korg clip CM-300 contact microphone (frequency response 0-60 kHz z) attached directly to the substrate where the bait was offered, 2) Jez Riley French C-Series contact microphone (frequency response 0-96 kHz) also attached directly to the substrate with the bait; and 3) Dodotronic Ultramic 384 kHz (frequency response 0-90 kHz), coupled to an Ipad connected to the Ultrasonic Software (Annex 1), directed at the ant during a distress call. The Jez and Korg microphones recorded ant sounds < 1mm away from the microphone (under baits or when held with forceps). Recordings with a Dodotronic microphone were taken at 5 mm from the immobile individual by forceps.

Acoustic analysis

We used previous information on the presence of stridulatory organs across ant genera (Golden and Hill 2016) to help distinguish sounds produced by stridulation and percussion. Given the different nature of the sounds, we used only stridulatory signals in the following analysis. However, we included information about percussion in the main results. We analyzed sound recordings using Raven Pro 1.5.2 Software (Cornell University Laboratory of Ornithology). We selected high-quality acoustic signals from our recordings by visually examining spectrograms and power spectra. From these, we estimated frequency and time features (Table 1). We defined a signal as monosyllabic when only one subunit of energy was emitted and disyllabic when two different subunits of energy were emitted in a specified interval. Temporal features were estimated from oscillograms, while frequency features from spectrograms (Fig. 2 and Fig. 2a). For the frequency estimates, we used the robust measures present in the Raven Pro 1.5.2 Software since many of the acoustic signals emitted did not present evident amplitude peaks to use the power-spectrum, as proposed by (Podos 1997) and (Zollinger et al. 2012). Spectrograms were derived with the following settings: Window type = Hamming, window size = 128, overlap = 50%. We edited spectrogram images using the *Seewave* package (Sueur et al. 2008b) for R (R Development Core Team 2022).

Table 1. Acoustic parameters and respective descriptions are taken from Software Raven Pro 1.5.2.

Acoustic parameter	Description
Duration	Signal duration time within the selection.
Number of pulses	The total number of pulses present in the acoustic signal.
Bandwidth	Difference between the highest and lowest frequency within the selection.
Lowest frequency	The lowest frequency within the selection.
Highest frequency	The highest frequency within the selection.
Frequency 5%	Frequency that divides the selection into two frequency ranges containing 5% and 95% of the energy.
Frequency 95%	Frequency that divides the selection into two frequency ranges containing 95% and 5% of the energy.
Peak frequency	The frequency at which the maximum power/peak frequency occurs within the selection.
1st frequency quartile	Timepoint that splits the selection into two ranges containing 25% and 75% of the energy within the section.

3rd frequency quartile	Timepoint that splits the selection into two ranges containing 75% and 25% of the energy within the section.

Data analysis

The analyses were performed using four datasets, one for each recorder and microphone combination: (1) ZOOM HN14 + Jez mic and (2) ZOOM HN14 + Korg for sounds on baits; and (3) Mix-Pre II + Ipad + Dodotronic and (4) Mix-Pre II + Jez mic for distress calls. Discriminant functions (LDA) were generated to assess the capacity of the recorded sounds to distinguish species. For each of these data sets, a function was generated using only spectral data (Spectral Model) and another function using the spectral + temporal data (Full model). We used only recordings with voucher specimens correctly identified to validate the models. All LDA functions were generated with 2/3 of the samples as a training set and using the remaining 1/3 for validation. Each model was repeated 100 times with randomization of the training and validation subassemblies, and the average of the 100 iterations was compared. We obtained the percentage of correct identifications for each test to construct a confusion matrix. Analyses were performed in R (R Core Team 2023) using the vegan package (Oksanen et al. 2022).

RESULTS

We collected and identified 70 species of ants distributed in 27 genera and eight subfamilies. Among these, 38 species from 15 genera and eight subfamilies exhibited acoustic signal emission during recruitment and *distress call*. However, only 28 species were used in the analyses (Table2). The remaining 10 species were recorded with a different combination of microphones and recorders during the testing period and adjustment of the recording protocol and were not included in the analyses. Independently of recording equipment, field recordings were affected by background noise. Even so, it was possible to distinguish acoustic signals confidently. Among the 28 species, 629 events were recorded, with 168 quality.

Species in the genera *Azteca*, *Camponotus*, *Labidus* and *Nylanderia* emitted percussive acoustic signals only during alarm or distress contexts. Percussive signals were primarily low frequency except for the species *Camponotus rapax*, *Camponotus femoratus* and *Azteca* sp. 2, which emitted frequencies in the ultrasonic range (above 20 kHz). *Labidus predator* presented longer percussive acoustic signals and a narrower bandwidth than the other species.

Ants from the subfamilies Ponerinae, Paraponerinae and Ectatominnae emitted acoustic signals during stress. Still, not during recruitment (Fig**.** 1**A**). Most of the acoustic signals from *Ectatomma*, *Neoponera* and *Odontomachus* species comprised a portion with lower energy followed by a portion with higher energy, mainly in the ultrasonic range.

Species in the subfamilies Myrmicinae and Pseudomyrmicinae emitted acoustic signals during recruitment and alarm situations. *Pseudomyrmex* recruitment signals were recorded by attaching the microphone to a host plant. The ants emitted low-frequency signals and recruited nestmates to protect the plant. In the context of distress calls, the acoustic signals presented higher frequencies and disyllable signals (Fig. 1**B, C, D**). The acoustic emissions of *Atta*, *Acromyrmex*, *Crematogaster*, *Pheidole*, *Mycetomoellerius* and *Solenopsis* species were produced by stridulation during recruitment behaviour or stress. These signals were composed of low or ultrasonic frequencies. We also observed the presence of disyllabic signals in species of *Atta*, *Acromyrmex*, *Crematogaster*, *Solenopsis,* and *Pheidole* in situations of alarm and recruitment, with frequencies below or above 20 kHz. In general, the acoustic signals showed remarkable diversity regarding the distribution of frequencies and duration of the signals, ranging from low bandwidth sounds with few harmonics, such as *Pheidole sp.7*, *Pheidole bioconstricta* and *Pheidole* sp. 60, to acoustic signals with ample bandwidth as in *Atta sexdens;* and harmonics above 20 kHz emitted by *Pheidole* sp. 38. refer to the supplementary files "SM01", the audios are available on SoundCloud (https://soundcloud.com/atta-amazonas/tracks).

Table 2. Summary of the occurrence and number of recording events of sounds emitted by ants used in the analysis according to each recording equipment and behaviour context. Some species were recorded in both behaviour contexts.

Behavioural contexts	Species	Dodotronic mic	Jezz mic	Korg mic
Recruitment call	*Atta laevigata*			75
	Camponotus femoratus			14
	Crematogaster brasilienses		47	
	Crematogaster sp1		22	
	Labidus predator			9
	Pheidole sp.30			12
	Pheidole sp.31		51	204
	Pheidole sp.32			13
	Pheidole sp.36		5	33
	Solenopsis sp.12		68	

	Solenopsis sp.13		34	
	Solenopsis sp.14		41	8
Distress call	*Atta laevigata*	57		18
	Atta sexdens	65	211	
	Crematogaster obscurata		142	
	Ectatomma brunneum	439	97	9
	Ectatomma lugens	271		
	Neoponera apicalis	115	9	
	Neoponera commutata	135	56	
	Odontomachus bauri	15		9
	Odontomachus haematodos	9	9	
	Odontomachus laticeps	4		
	Paraponera clavata		4	
	Mycetomoellerius sp.1		99	
	Pheidole sp.34		51	
	Pheidole sp.38		111	
	Pseudomyrmex concolor		175	
	Solenopsis sp.12		361	
	Solenopsis sp.15		87	

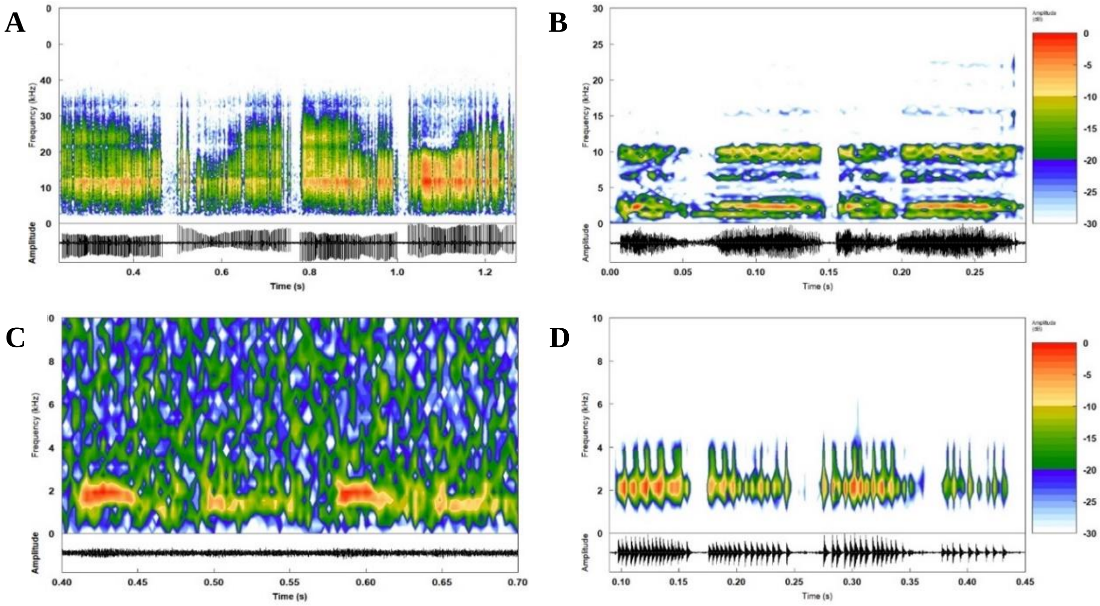


Figure 1. Figure 1. Spectrograms and oscillograms of four acoustic signals. **A)** *Ectatomma brunneum* recorded with Dodotronic mic + iPad, **B)** *Pheidole* sp. 34, recorded with the Jez mic and Mix-Pre II during distress calls, showing disyllabic signals, **C)** *Pheidole* sp. 31 recorded with Korg mic and Zoom F4 recorder, showing disyllabic signals, and **D)** *Solenopsis* sp. 13 recorded with Jez mic and Mix-Pre II recorder, during recruitment to a food source. Spectrograms show different frequency scales for better visualization.

We detected a considerable variation in species identification accuracy according to the different equipment combinations and behavioural contexts. For example, the accuracy of species recordings during recruitment using the Jez microphone ranged from ~30% to ~100% using only spectral data (Fig. 2**A).** Still, with temporal combined with spectral data, the accuracy ranged from ~36% to ~100% (Fig, 2**B**). Overall, based only on the acoustic signal, the LDA model correctly identified the species between 30% and 100% of the tests. The model including only spectral data, identified *Ectatomma brunneum* in ~100% of cases. *Mycetomoellerius* sp. identification showed a lower accuracy of ~30-35% in using both data sets (Fig. 2**A**, **B**).

The accuracy of recruitment recordings using the Korg microphone ranged from 0 (zero) to ~80% using only spectral data (Fig. 2**C**). With the combination of temporal data, the accuracy increased from ~47% to 89% of the tests (Fig. 2**D**). Using only spectral data, it was not possible to identify the species *Pheidole* sp 32. The sounds of this species were indistinguishable from *Acromyrmex subterraneus*. The *Pheidole* sp 30 also had a lower accuracy, being correctly identified only in ~4% of the cases. All species, except *Pheidole* sp. 36, showed higher identification accuracy using the full dataset (spectral + temporal) than only spectral parameters data (Fig. 2**C**, **D**).

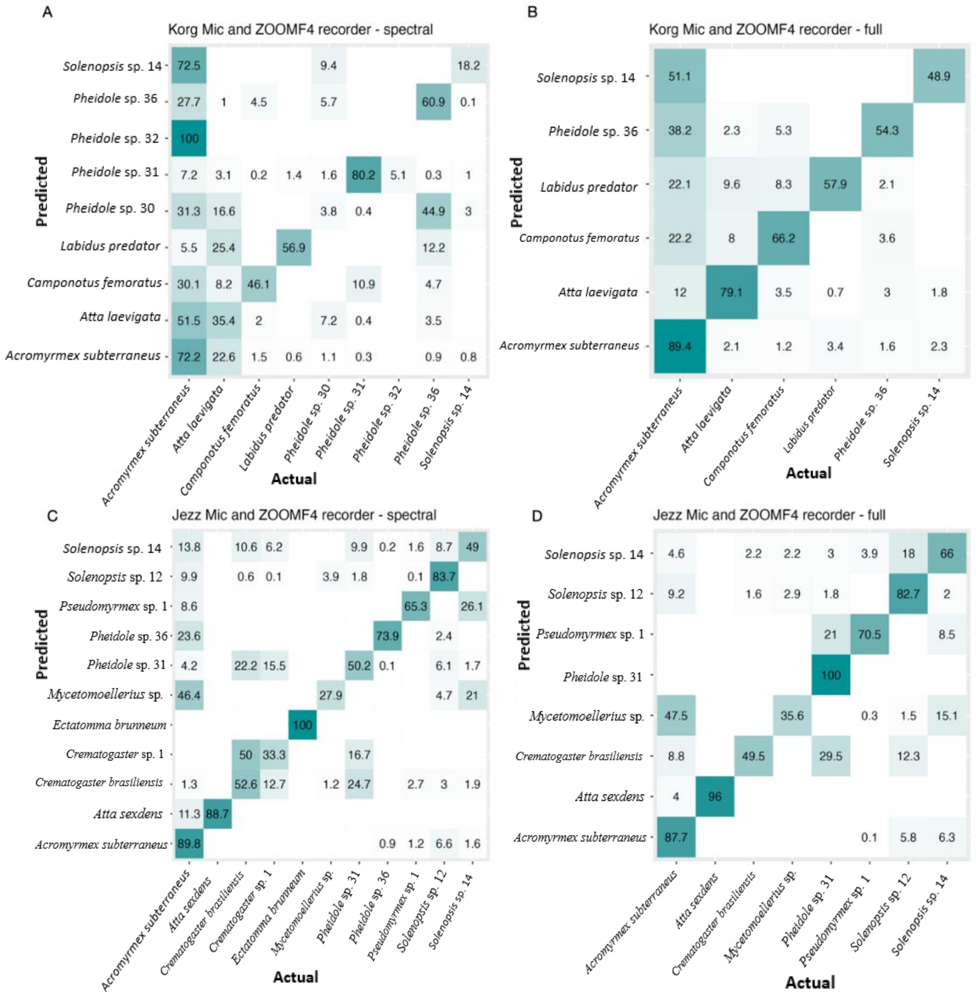
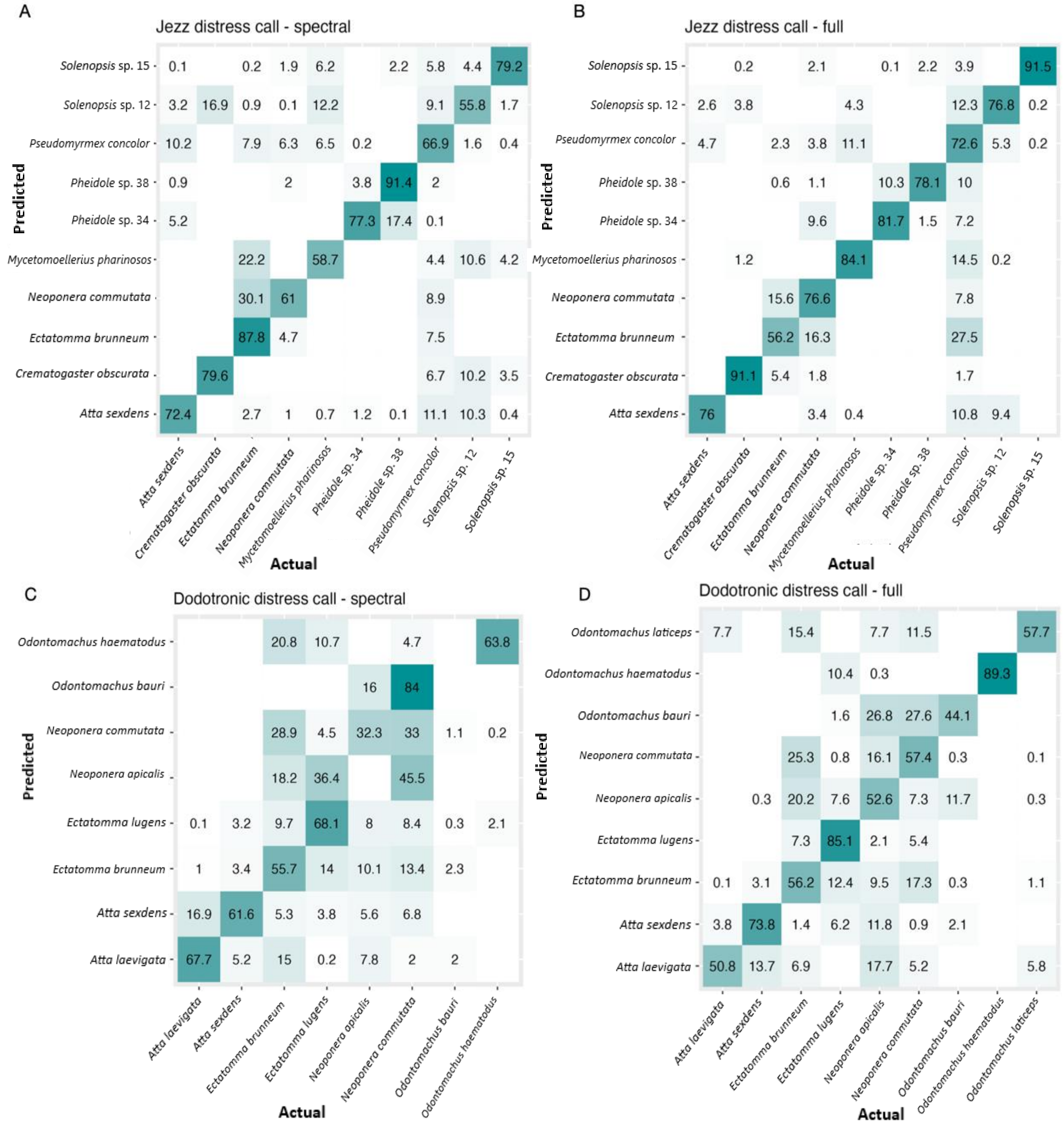


Figure 2. Matrices with the results of Linear Discriminant Analysis for the four models created for each equipment combination using only spectral data (**A** and **C**) and spectral + temporal data (**B** and **D**). The observed species names are in columns, while predicted names are in rows. Therefore, the values on the diagonal are correct predictions, and off-diagonal values are incorrect predictions

The accuracy of identification using the distress call recordings using a Dodotronic microphone ranged from 0 (zero) to ~80% using only spectral data (Fig, 3**A**). However, with temporal data combined with spectral data, the accuracy ranged from ~40% to ~90% (Fig. 3**B**). The most notable cases were *Neoponera apicalis* and *Odontomachus bauri*. The model based on spectral data with the Dodotronic microphone could not discern the distress call of these species, confusing with the sounds of *Neoponera commutata*, *Ectatomma lugens* and *Ectatomma brunneum* (Fig. 3**A**). However, including temporal parameters, the model's accuracy increased to ~50% for *N. apicalis* and ~40% for *O. bauri* (Fig. 3**B)**. Other species showed the opposite pattern. The model using only spectral data correctly identified *Atta laevigata* and *Ectatomma brunneum* in ~68 and ~56% of cases, respectively, but dropped to ~50 and ~54% using spectral data combined with temporal data.

Compared to the Dodotronic microphone, the Jez microphone showed higher accuracy in distress call recordings, which ranged from 55% to ~80% using only spectral data (Fig. 3**C**). When combining temporal and spectral data, accuracy ranged from ~56% to ~93% (Fig. 3**D**). For example, the accuracy of *Solenopsis* sp. 15. based only on spectral data was ~80% but increased to ~90% when combined with temporal data. However, using only spectral data, the model correctly identified *Pheidole* sp 38 in ~90% and *Ectatomma brunneum* in ~84% of cases. Still, the percentage dropped to ~70% and ~56% using spectral data combined with temporal data.

Overall, adding temporal data to the models increased the correct species identification based only on their respective acoustic signals. In addition, recruitment recordings were generally more helpful for species identification than distress calls (Figure 4). The Jez and Korg microphones performed better than the Dodotronic mic (Fig. 4). The best equipment combination during the distress call context tested was the Jez contact mic and Mix-Pre II recorder. Using this equipment setup, it was possible to identify 50% of the tested species with at least 80% accuracy.



A — Jezz distress call - spectral

	Atta sexdens	*Crematogaster obscurata*	*Ectatomma brunneum*	*Neoponera commutata*	*Mycetomoellerius pharinosos*	*Pheidole* sp. 34	*Pheidole* sp. 38	*Pseudomyrmex concolor*	*Solenopsis* sp. 12	*Solenopsis* sp. 15
Solenopsis sp. 15	0.1		0.2	1.9	6.2		2.2	5.8	4.4	79.2
Solenopsis sp. 12	3.2	16.9	0.9	0.1	12.2			9.1	55.8	1.7
Pseudomyrmex concolor	10.2		7.9	6.3	6.5	0.2		66.9	1.6	0.4
Pheidole sp. 38	0.9			2		3.8	91.4	2		
Pheidole sp. 34	5.2					77.3	17.4	0.1		
Mycetomoellerius pharinosos			22.2		58.7			4.4	10.6	4.2
Neoponera commutata			30.1	61				8.9		
Ectatomma brunneum			87.8	4.7				7.5		
Crematogaster obscurata		79.6						6.7	10.2	3.5
Atta sexdens	72.4		2.7	1	0.7	1.2	0.1	11.1	10.3	0.4

B — Jezz distress call - full

	Atta sexdens	*Crematogaster obscurata*	*Ectatomma brunneum*	*Neoponera commutata*	*Mycetomoellerius pharinosos*	*Pheidole* sp. 34	*Pheidole* sp. 38	*Pseudomyrmex concolor*	*Solenopsis* sp. 12	*Solenopsis* sp. 15
Solenopsis sp. 15		0.2		2.1		0.1	2.2	3.9		91.5
Solenopsis sp. 12	2.6	3.8			4.3			12.3	76.8	0.2
Pseudomyrmex concolor	4.7		2.3	3.8	11.1			72.6	5.3	0.2
Pheidole sp. 38			0.6	1.1		10.3	78.1	10		
Pheidole sp. 34				9.6		81.7	1.5	7.2		
Mycetomoellerius pharinosos		1.2			84.1			14.5	0.2	
Neoponera commutata			15.6	76.6				7.8		
Ectatomma brunneum			56.2	16.3				27.5		
Crematogaster obscurata		91.1	5.4	1.8				1.7		
Atta sexdens	76			3.4	0.4			10.8	9.4	

C — Dodotronic distress call - spectral

	Atta laevigata	*Atta sexdens*	*Ectatomma brunneum*	*Ectatomma lugens*	*Neoponera apicalis*	*Neoponera commutata*	*Odontomachus bauri*	*Odontomachus haematodus*
Odontomachus haematodus			20.8	10.7		4.7		63.8
Odontomachus bauri					16	84		
Neoponera commutata			28.9	4.5	32.3	33	1.1	0.2
Neoponera apicalis			18.2	36.4		45.5		
Ectatomma lugens	0.1	3.2	9.7	68.1	8	8.4	0.3	2.1
Ectatomma brunneum	1	3.4	55.7	14	10.1	13.4	2.3	
Atta sexdens	16.9	61.6	5.3	3.8	5.6	6.8		
Atta laevigata	67.7	5.2	15	0.2	7.8	2	2	

D — Dodotronic distress call - full

	Atta laevigata	*Atta sexdens*	*Ectatomma brunneum*	*Ectatomma lugens*	*Neoponera apicalis*	*Neoponera commutata*	*Odontomachus bauri*	*Odontomachus haematodus*	*Odontomachus laticeps*
Odontomachus laticeps	7.7		15.4		7.7	11.5			57.7
Odontomachus haematodus				10.4	0.3			89.3	
Odontomachus bauri				1.6	26.8	27.6	44.1		
Neoponera commutata			25.3	0.8	16.1	57.4	0.3		0.1
Neoponera apicalis		0.3	20.2	7.6	52.6	7.3	11.7		0.3
Ectatomma lugens			7.3	85.1	2.1	5.4			
Ectatomma brunneum	0.1	3.1	56.2	12.4	9.5	17.3	0.3		1.1
Atta sexdens	3.8	73.8	1.4	6.2	11.8	0.9	2.1		
Atta laevigata	50.8	13.7	6.9		17.7	5.2			5.8

Figure 3. Matrices with the results of Linear Discriminant Analysis for the four models created for each equipment combination using only spectral data (**A** and **C**) and spectral + temporal data (**B** and **D**). The models were created using only distress call records. The observed species names are in columns, while predicted names are in rows. Therefore, the values on the diagonal are correct predictions, and off-diagonal values are incorrect predictions.

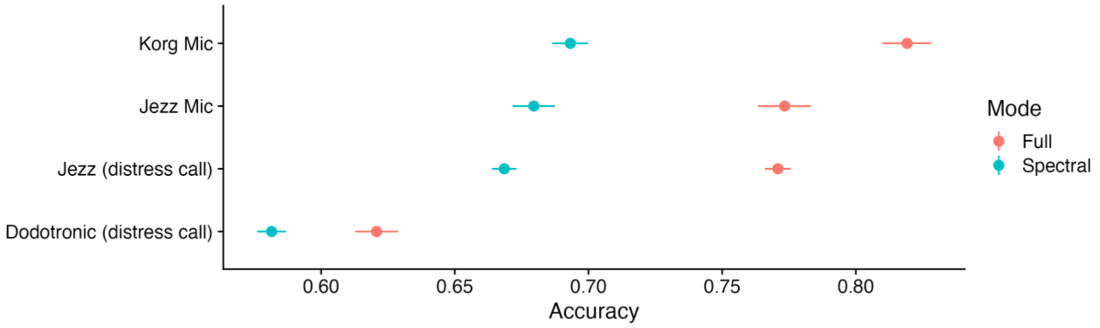


Figure 4. Overall accuracy of Linear Discriminant Analysis for the four models created for each equipment using only spectral data and spectral + temporal data (distress call) for recordings during recruitment and distress call. The lines indicated the 95% CI based on 100 bootstrap permutations.

DISCUSSION

Understanding the acoustic communication of ants in tropical forests is crucial, given the vast diversity of ant species (Andrade-Silva et al. 2022) and their significant roles in ecosystem functioning (Del Toro et al. 2015). Acoustic signals from ant species offer a promising tool for integrative biology studies and passive acoustic monitoring (PAM) in soil environments (Ganchev 2007).

This paper introduces an affordable methodology for collecting these acoustic signals and evaluates the accuracy of species identification using the collected data. We compared three microphones and two recorders under different behavioural conditions, finding that the Korg and Jez contact microphones captured higher-quality signals across a wider range of emitted frequencies compared to the Dodotronic directional microphone. Furthermore, incorporating temporal data substantially enhanced model accuracy, assessed as the proportion of correct identifications relative to the total number of ants recorded. The success rate improved significantly when analysing both spectral and temporal parameters (Full) versus solely spectral parameters (Spectral). On average, species identification based on the collected audio signals achieved a success rate of 55 to 90%. Future research into the spectro-temporal characteristics of these signals may uncover previously underestimated relationships in various unexplored ant behaviours.

Equipment performance and signal identification

We recorded ants that produce stridulatory sounds, as well as ants that lack a stridulatory organ and produce percussive sounds. Most percussion recordings were made in alarm situations, in species usually associated with plants in the *Azteca* and *Camponotus* genera and army ant species in the *Eciton* and *Labidus* genera. We observed subtle leg movements and rapid movements of the gaster on the substrate, and the ants seemed to modulate the emission of acoustic signals during locomotion behaviour observed near the contact microphone. Some species, such as *Azteca sp* 2, showed ultrasonic frequency values (above 20 kHz), reported here for the first time. We suggest the percussive acoustic signals and the behaviours that modulate thes signals should be considered in future studies of the evolution of acoustic communication in ants (Golden and Hill 2016).

The emission of acoustic signals by poneromorph ants occurs mainly during defensive behaviour or *distress calls* (Markl 1965, Ferreira et al. 2014), emitted in possible situations of stress caused by predation or disturbance in the nest (Markl 1973). In this group, the OS morphology, the presence of acoustic signals and their characteristics have been used as tools to aid in the separation of species into complexes of sympatric species, as observed in the species complex of *Pachycondila apicalis*, current *Neoponera apicallis* (Ferreira et al. 2010, Ferreira et al. 2014) and in the *Ectatomma ruidum* species complex (Peña Carrillo et al. 2022). The recorded distress call acoustic signals were similar to those found in the literature, with a long duration and one or two energy subunits depending on the caste (Pavan et al. 1997; Ferreira et al. 2010; Ferreira et al. 2014; Castro et al. 2015; Medeiros 2017; Peña Carrillo at al. 2022). With a combination of temporal and spectral parameters, our models could predict the correct species among different genera, such as *Ectatomma*, *Odontomachus*, and *Neoponera*.

We also recorded the acoustic signal of *Ectatomma brunneum*, which is reported here for the first time. The signal duration and frequency were higher than in the species *Ectatomma tuberculatum*, which has been previously recorded. We detected a shorter signal in *E. tuberculatum*, which contrasts with the results found by (Pavan et al. 1997), although they also found that the species had signals with disyllabic patterns. We also recorded the specialist species *Neoponera commutata* for the first time; it presented both disyllabic and monosyllabic signals. This difference in signal type may be related to caste, as observed in *Neoponera apicalis* (Medeiros 2017). In Ponerinae, acoustic emission within the colony has been observed between individuals from the same nest (Ferreira et al. 2014), but we did not detect acoustic signals from Ponerinae during foraging. More studies are needed in other behavioural contexts, such as intraspecific and interspecific interactions, to understand this behaviour in this subfamily, as well as studies at the community level.

Stridulation and percussive sounds

Percussion and stridulation are present in several lineages in ants; they are mechanisms that generate vibration and result in the emission of acoustic signals (Hickling and Brown 2000). The vibration of the ant's body combined with the release of chemical signals makes the reach of chemical information more efficient (Hölldobler et al. 1996). Recently, it was demonstrated that noise disturbances could impact leafcutter ants and adjust their foraging behaviour in the presence of noise (Byrne et al. 2022). In addition, two species of drumming ants and myrmecophytes of the genus *Camponotus* exhibited distinct acoustic signals in response to mechanical disturbances. The signals had components transmitted through the substrate and the air, and these alarm signals may assist in intraspecific and intercolonial communication (Bota et al. 2022). However, we still know very little about the functions or advantages of species that stridulate during food source recruitment behaviour in the wild. We still don't understand whether dominant species acoustically signal their presence at food sources. As the species *Crematogaster scutellaris*, which emits disyllabic signals, modulate frequency and time parameters during stridulation in behavioural contexts of food restriction and feeding (Masoni et al. 2021). Our results suggest that species that recruit and monopolize resources routinely emit acoustic signals, and the majority (~90%) emit signals that can be discerned between species. It is possible that the complexity in acoustic repertoire of recruiting ants can signal to other species their investment in resource monopolization. However, the function of these acoustic signals between dominant and subordinate species needs to be investigated in greater detail.

Distress calls and species complexes

The use of recordings to distinguish species according to distress calls has been less investigated in the Myrmicinae than Ponerinae (Ferreira et al. 2010; Ferreira et al. 2014; Peña Carrillo at al. 2022). Our distress call recordings among Myrmicinae species showed disyllabic signals with two energy subunits, also observed within the subfamily Ponerinae. This can be explained by the movement of the SO or by differentiation across ant castes. When considering the equipment used, the Jez contact microphone facilitated recording distress call signals among species in the Myrmycinae. We identified species through temporal and spectral data on 55% to ~90% of attempts. The Dodotronic directional microphone was most suitable for studies to capture acoustic distress call signals in Ponerinae species, as it can capture ultrasonic signals. However, it had the lowest accuracy, and we successfully identified species on between 40% and ~80% of attempts. Among the microphones used, the Jez contact microphone seemed a good alternative for recording distress call signals in Ponerinae and Myrmicinae. It was successful on 55% to ~90% of identification attempts. The Korg and Jez contact microphones are also great options for behavioural studies, as they are small and less invasive. The Jez microphone can capture more limited ultrasonic frequencies than the Dodotronic. It can be used to make recordings directly in the field or the laboratory, allowing us to explore more behavioural contexts in stridulating and percussive ants.

Myrmicinae diversity and recruitment signals

The ant subfamily Myrmicinae is the most diverse in the Neotropical region (Barbero et al. 2015). We also found a great diversity of acoustic signals during recruitment behaviour to a food source, characterized by low frequencies and different harmonics. Compared to distress call signals, most recruitment signals at baits were monosyllabic, i.e. with only one subunit of energy. We found similarities in the spectro-temporal characteristics of acoustic signals between species of *Pheidole, Solenopsis* and *Crematogaster*. We also found similarities between subfamilies. In the Pseudomyrmicinae, for example, we recorded ants during mate recruitment for plant protection and observed acoustic similarities with species from the subfamily Myrmicinae. Thus, it is possible to observe similarities in acoustic signals between species from different subfamilies during recruitment to food sources and recruitment to protect the nest. This suggests a possible convergence in acoustic signals according to the behavioural contexts used.

Temporal parameters in species delimitation

Past research has shown that in acoustic signalling by insects *other* than ants, temporal parameters are more important than frequency parameters, and minute changes in these parameters can aid in distinguishing genera and species (Drosopoulos and Claridge 2005; Kowalski and Lakes-Harlan 2011). Our results corroborate this pattern and explain why including temporal parameters improved the models' discernment of species. Using temporal parameters also improved the accuracy of the models for all equipment combinations. The combination of temporal parameters with spectral parameters seems to be more representative of ants in general. However, we did not estimate more refined time parameters such as the length of intervals between signals, the average interval between pulses, or the pulse repetition rate (Ferreira et al. 2010). Including other temporal parameters should further improve recording performance in the species delimitation process and provide more information about the role of acoustic signals in different behavioural contexts. Our research suggests that temporal parameters are crucial in delimiting subfamilies and genera of ant species.

CONCLUSIONS

Our study demonstrates that acoustic signals in ants can serve as a valuable tool for species identification and understanding their ecological interactions, particularly in tropical forests. By integrating spectral and temporal data, we significantly improved identification accuracy during key behaviours such as recruitment and distress calls. Our findings contribute to the existing body of knowledge by revealing new acoustic characteristics in certain species and highlighting the adaptive convergence of signals across different subfamilies. This research not only enhances our understanding of ant communication but also sets the groundwork for future studies to explore the ecological roles of these signals, encouraging further investigation into the relationship between acoustic behaviour and species interactions in diverse environments.

ACKNOWLEDGEMENT

The research was funded by CNPQ, UFAM, the Oregon Arts Commission, and the Oregon Community Foundation. We Thanks to the Projeto LABVERDE and Anthony Brisson for all the support. Cornell Lab kindly provided Raven Pro.1.5.2 acoustic analysis software. This work emerged from discussions of the ATTA (Amplifying the Tropical Ants) group. FBB is continuously supported by a CNPq grant (#313986/2020-7).

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Capítulo 2

VALE E, SHONBERG L, VASCONCELOS A, BOBROWIEC P, BACARRO F (2024) **Relationships between numerical dominance and morphological characteristics in the acoustic signals of terrestrial ants.**

Relações entre dominância numérica e características morfológicas nos sinais acústicos de formigas terrestres

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RESUMO

As formigas utilizam sinais acústicos, emitidos por percussão corporal ou estridulação, para comunicação. No entanto, pouco se sabe sobre o papel desses sinais na dominância por recursos alimentares e na competição entre espécies. Este estudo investigou a influência da dominância numérica e das características morfológicas do aparato estridulatório na emissão de sinais acústicos e nos parâmetros espectro-temporais de formigas da Amazônia Central. Foram coletadas amostras de aproximadamente 18 espécies dominantes, principalmente da subfamília Myrmicinae, e 8 espécies subordinadas, predominantemente da subfamília Ponerinae. Nossos resultados indicam que, durante interações de dominância por recursos alimentares, as formigas subordinadas emitem, em média, um maior número de sinais acústicos em comparação às dominantes. Os parâmetros espectrais dos sinais diferem entre as espécies dominantes e subordinadas, enquanto os parâmetros temporais mostraram-se semelhantes. Na análise de sinais acústicos de "distress call", encontramos uma correlação significativa entre a altura do gáster e o número de pulsos, mas não com a média do intervalo de pulsos ou a distância entre as cristas. Com esses achados, esperamos contribuir para a compreensão do comportamento de dominância nas formigas e da evolução da comunicação acústica em Hymenoptera.

Palavras-Chave: estridulação, formigas, Amazônia, comportamento, recrutamento

Relationships between numerical dominance and morphological characteristics in the acoustic signals of ground ants

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ABSTRACT

Ants emit acoustic signals through percussion with the body or stridulation through the stridulatory apparatus. Although studies have analysed behaviour and used acoustic signals as a diagnostic tool to identify ant species, we still don't know whether the emission of acoustic signals plays a role in dominance over food resources, and consequently in competition for resources. In this research, I investigated how the numerical dominance and morphology of the stridulatory apparatus influence the emission of acoustic signals and the spectro-temporal parameters of ants from the Central Amazon. Around 18 species of dominant ants and 8 species of submissive ants were collected. The dominant ants belonged to the Myrmicinae subfamily, and the submissive ants belonged mainly to the Ponerinae subfamily. Our results show that during dominance behaviour due to food resources, subordinate ants emit a greater number of acoustic signals in the media compared to dominant ants. We found that the spectral perturbations of the signals vary between dominant and submissive species, but the temporal parameters are similar in this context. When analysing the distress-call acoustic signals, we clearly observed the relationship between the morphological characteristic gaster height and the temporal parameter number of pulses, but not in relation to the average pulse interval and the distance between the crests. Finally, we hope to contribute to studies on dominance behaviour and the presence of acoustic signals, as well as studies on the evolution of acoustic communication in ants.

Keywords: stridulation, ants, Amazonia, behaviour, recruitment

INTRODUÇÃO

Ant communication is multimodal; that is, ants can communicate through various mechanisms, including chemical, tactile, and acoustic signals (Markl 1965; Hölldobler 1999; Carlos et al. 2013). The best-known form of intraspecific communication in ants involves the emission of volatile chemical substances, soluble substances, and touch, collectively known as pheromones (Hölldobler and Wilson 1990). Recruitment pheromones are deposited on the ground in the form of trails, which indicate the presence and location of food (Hölldobler and Wilson 1990). Furthermore, it is understood that the emission of acoustic signals can enhance the propagation of signals mediated by chemical pheromones (Hölldobler 1978). During worker recruitment, ants may produce acoustic or stridulatory signals to complement or modify the information conveyed by chemical signals (Hölldobler et al. 1978; Baroni Urbani et al. 1988; Roces et al. 1993).

Acoustic signals can be generated through various mechanisms, including percussion (drumming behaviour), where certain parts of the body strike the substrate, or through stridulation, which occurs when there is a specialised apparatus, such as projections of chitinous structures (Hölldobler and Brown 2000). Stridulation is defined as the production of sound through the friction of differentiated regions of the exoskeleton (Leston and Pringle 1963). In ants, this frictional movement within the stridulatory apparatus is facilitated by the up-and-down motion of the gaster, resulting in the generation of acoustic signals known as chirps (Ewing 1989; Hickling and Brown 2000).

The morphology of the stridulatory apparatus comprises two main components: a scraper (*plectron*), located on the edge of the post-petiole or on the first segment of the gaster, depending on the species, and a modified structure (*pars stridens*) situated at the anterior end of the first gastric tergite. The *pars stridens* features cuticular ridges arranged in thin parallel layers known as striae (Figure 5) (Hölldobler and Wilson 1990; Yao et al. 2018). On the *pars stridens*, there are tegumental reliefs referred to as pillars, which serve as support structures with a sound-amplifying function (Álvarez 2009). Additionally, the presence of expanded air sacs within the gaster, in contact with the outer cuticle, further aids in sound amplification, as observed in *Solenopsis richeteri* (Hickling and Brown 2000).

There is evidence of a correlation between body size and the size of the pars-stridens, indicating that larger ant species possess larger *pars-stridens*. However, the average size of the ridges on the *pars-stridens* does not seem to depend on the overall size of the ant, as observed in certain Myrmicinae species (Castro et al. 2015). This morphological variation appears to influence the frequencies produced, which can range from low frequencies (< 20 kHz) to high frequencies (> 20 kHz), potentially reaching the ultrasound range. Nevertheless, few studies have examined the emission of acoustic signals at frequencies above 20 kHz in ants (Esperson 1994; Pavan et al. 1997).

Initially, stridulation in ants was thought to serve as a rescue signal, particularly in ground-dwelling species (Markl 1973; Ruiz et al. 2006; Hunt and Richard 2013). However, this hypothesis was not substantiated following a phylogenetic reconstruction of the ancestral state and the mapping of the stridulatory apparatus's presence. The proportion of ants exhibiting the stridulatory apparatus is higher among primarily arboreal species and has evolved independently at least five times across the subfamilies Pseudomyrmicinae, Myrmicinae, Ecatatominae, Paraponerinae, and Ponerinae (Golden and Hill 2016). Therefore, it is crucial to explore the emission of acoustic signals in various environments and behavioural contexts.

Ants are social insects that perform various tasks in cooperation, enhancing the foraging efficiency of their colonies (Oster and Wilson 1978). Foraging behaviour is influenced by multiple factors, including the availability of food, its spatial and temporal distribution, accessibility, the carrying capacity of individual workers, and the dominance relationships among ant species (Levings and Traniello 1981; Schoener 1971; Baccaro et al. 2012; Viana-Bailez and Endringer 2016). Ants can forage in three primary ways: 1) individually, where workers forage independently; 2) through recruitment, where workers search for food alone but enlist other ants for collection; and 3) specialised foraging, in which workers hunt in groups or parasitise other ant colonies (Oster and Wilson 1978).

Foraging through recruitment and the emission of acoustic signals has primarily been observed in ants of the genera *Atta* (fungus-growers) and *Aphaenogaster*, particularly during the recruitment of other individuals, leaf cutting, nest migration, agonistic encounters, and parental care of pupae (Mark 1965; Mark 1967; Baroni-Urbani et al. 1988; Roces and Hölldobler 1996; Rauth and Vison 2006). Additionally, some ant species can receive acoustic signals and respond to other mutualistic or parasitic insects (Barbero et al. 2009; Cassaci et al. 2014; Giulio et al. 2015; Schönrogge et al. 2016; Lin et al. 2019; Fattorini and Giulio 2021).

Competition and resultant behavioral dominance is a widely debated topic in ant ecology (Hölldobler and Wilson 1990; Cerdá et al. 2013). Behavioral dominance reflects an ant's ability to fight, recruit individuals, and monopolize food resources (Schoener 1983; Drews 1993). Among recruiting ants, three hierarchical levels of competition have been identified (Vespsäläinen and Pisonski 1982): 1) dominants, which are aggressive species that engage in mass recruitment; 2) occasional dominant or subdominant species, which can monopolize resources only in the absence of dominants; and 3) subordinates, which are non-aggressive species that primarily defend their nests. While numerous studies have demonstrated hierarchical dominance relationships in ants (Vepsäläinen and Pisarski 1982; Fellers 1987; Savolainen and Vepsäläinen 1988; Andersen 1992, Andersen 1997; Sanders et al. 2007; Baccaro and Souza 2007), it remains unclear how these dominance patterns regarding food resources influence the emission of acoustic signals.

The reception of acoustic signals in ants remains poorly understood. For a long time, it was believed that ants received acoustic signals transmitted solely through the substrate via structures located on the tibiae known as cord organs (or cordotonals), which have also been identified in other insect orders (Markl 1965; Roces et al. 1993; Field and Matheson 1998; Roces and Tautz 2001; Devetak and Delakorda 2004). However, it is likely that ants also receive acoustic signals through the air, via specialized trichoid sensilla (Dumpert 1972). These sensilla are predominantly located on the apical segments of the ants' antennae (Hashimoto 1990; Hickling and Brown 2000; Renthal et al. 2003).

The sensilla are cuticular projections responsible for receiving various stimuli and can serve multiple functions depending on their morphological structure. They may act as cuticular hydrocarbon or gustatory receptors (*basiconic sensilla*), hydroreceptors, thermoreceptors, mechanoreceptors (*coelocapitular and trichoid sensilla*), and chemoreceptors (*trichoid sensilla curvata* and *sensilla ampulacea*) (Barsagade et al. 2013; Dumpert 1972; Kleineidam et al. 2000; Renthal et al. 2003; Ozaki et al. 2005).

Although it is known that these morphological structures are involved in the production and possible reception of acoustic signals in ants, there remains a significant gap in research regarding how the spectro-temporal parameters of these signals are influenced by the morphological characteristics of stridulating ants. Furthermore, the majority of studies linking the emission of acoustic signals to behaviour and morphology have primarily focused on temperate and subtropical regions (Hölldobler 1998; Hickling and Brown 2000; Jackson and Ratnieks 2006; Ferreira et al. 2010; Mankin and Benshemesh 2006; Pielström and Roces 2012). Therefore, our objectives are to investigate: (1) how numerical dominance behaviour and morphological characteristics influence the emission and spectro-temporal parameters of acoustic signals; and (2) how morphological characteristics influence the spectro-temporal patterns of acoustic signals emitted by soil ant species in Central Amazon.

MATERIAL AND METHODS

Study area

The samples were collected over 15 days in August and September 2023 at the Adolpho Ducke Forest Reserve (2°57'42“S 59°55'40” W) (Figure 1), in the municipality of Manaus, AM, Brazil. The 10,000-hectare Ducke Reserve is located an area of non-flooded (terra firme) forest. The reserve has a system of trails that form a grid of 25 km² (5 x 5 km). The grid has 30 permanent plots spaced systematically at 1 km intervals, 23 of which are in areas close to watercourses (Baccaro et al. 2008).

The climate of the reserve is classified as tropical humid, with relative humidity of 75-86% and annual rainfall of between 1,750 and 2,500 mm. The average annual temperature is 26 °C. The elevation of the terrain varies from 80 to 140 m, with shallows frequently flooded during the rainy season. The region is covered by low-altitude tropical rainforest, with a closed canopy (Baccaro et al. 2008).

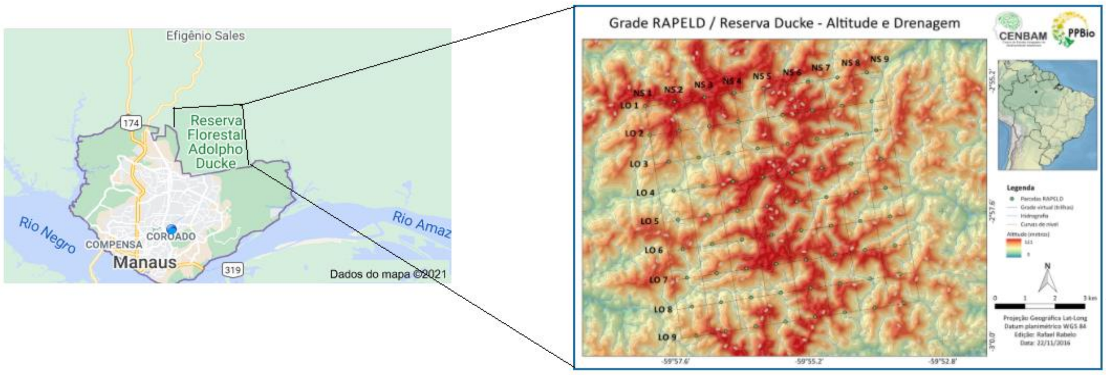


Figure 1. Location of the Ducke Reserve, with the city of Manaus to the south. The trail system is shown in the figure to the right Source: www.ppbio.inpa.gov.br

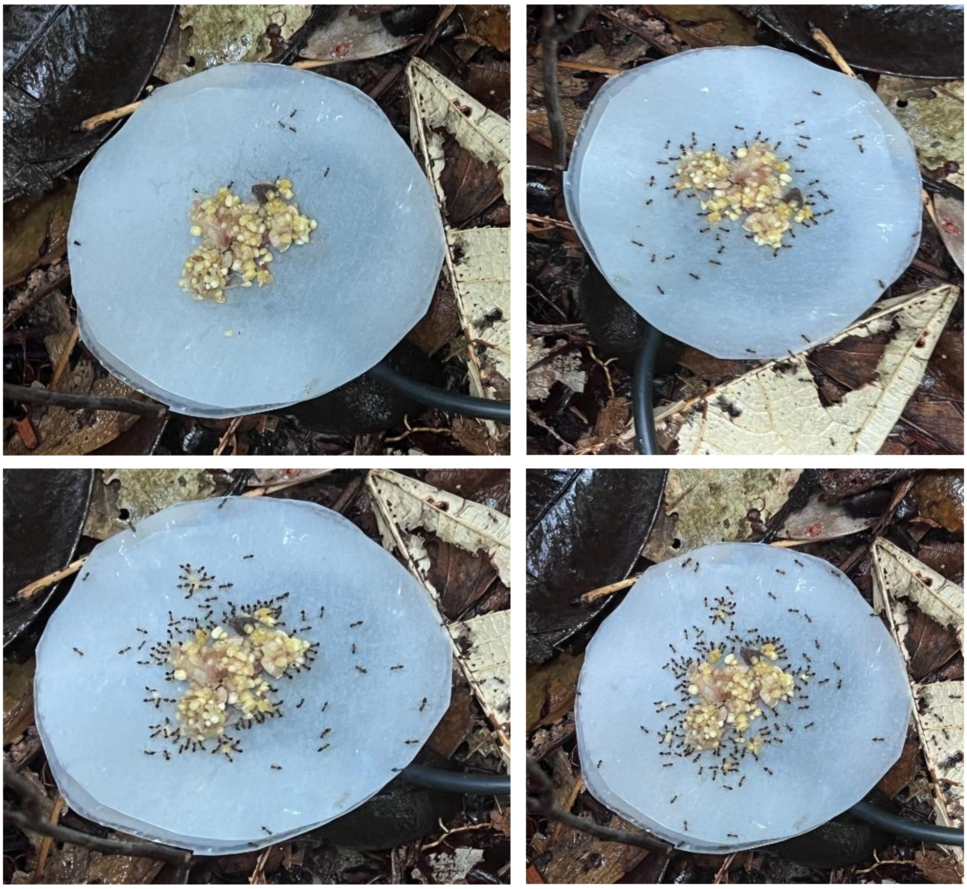


Figure 2. Photographs of the arenas with the attractive bait (flour + sardines) during the dominance behavior of the species *Crematogaster tenuicula*.

Behavioral observations

We used baits to evaluate the relationship between dominance behavior and the emission of acoustic signals by ants (objective 1). Each bait was observed individually for up to 2 hours. First, I attached the microphone to the substrate and positioned a piece of baking paper 10 cm in diameter above it, where the bait was placed (~10 g of flour and sardines in a 1:1 ratio) (Figure 2). We sampled 93 baits which were placed every 20 meters in 200 m transects. As the distribution of ants is influenced by the relief of the terrain (Vasconcelos et al. 2003), the baits were distributed on plateaus, slopes and shallows to maximize the number of species investigated.

The contexts of interaction between ant species were recorded from the presence of the first ant on the bait. The behavior on the baits was monitored for 2 hours with the aid of video recordings using a Canon Powershot camera, to avoid the effect of the researcher's presence near the bait. During the recording, we counted the number of individuals of each species present at the bait. We used data from the literature (Baccaro and Souza 2007; Baccaro 2015; Utta 2017) and the results of our baits to classify the ant species as dominant or subordinate. The species considered dominant were those that dominated at least 25% of the baits that occurred and recruited on average more than 20 workers per bait (Andersen 1992 Baccaro et al. 2010). Baits were dominated when only one species was feeding on the bait, with at least 20 individuals. Although these criteria use numerical descriptors, numerical dominance is positively related to behavioral dominance (Fellers 1987, Andersen 1992, Parr 2008).

Recording and reading of acoustic parameters

The ants' acoustic signals were recorded using a MIX-PRE II recorder with a sample rate of 96 kHz and a sample size of 32 bits, coupled to a JEZ piezo contact microphone, which responds to frequencies of up to 70 kHz. For each recording, visual representations of the recordings (spectrogram and sonogram) were projected in Raven Pro 1.6 software (Cornel Bioacustics Lab). Only good quality recordings were used for analysis.

For each recording, eight spectral parameters were measured: bandwidth, maximum frequency, frequencies at 5%, 25%, 50%, 75%, 1st frequency quartile, 3rd frequency quartile (Figure 3). Four temporal parameters were measured: signal duration (chirp), interval between chirps, average interval between chirps and number of pulses of each chirp (Medeiros 2017) (Figure 4). The spectro-temporal parameters are described in Table 1.

To answer objective 2, the captured ants were recorded individually in the laboratory in the context of a distress-call to better associate the acoustic signal with the sending individual. To do this, I immobilized the mesosoma region by holding it with tweezers, leaving the gastral region free and positioning the individual over the microphone. A minimum of 6 individuals per species were used.

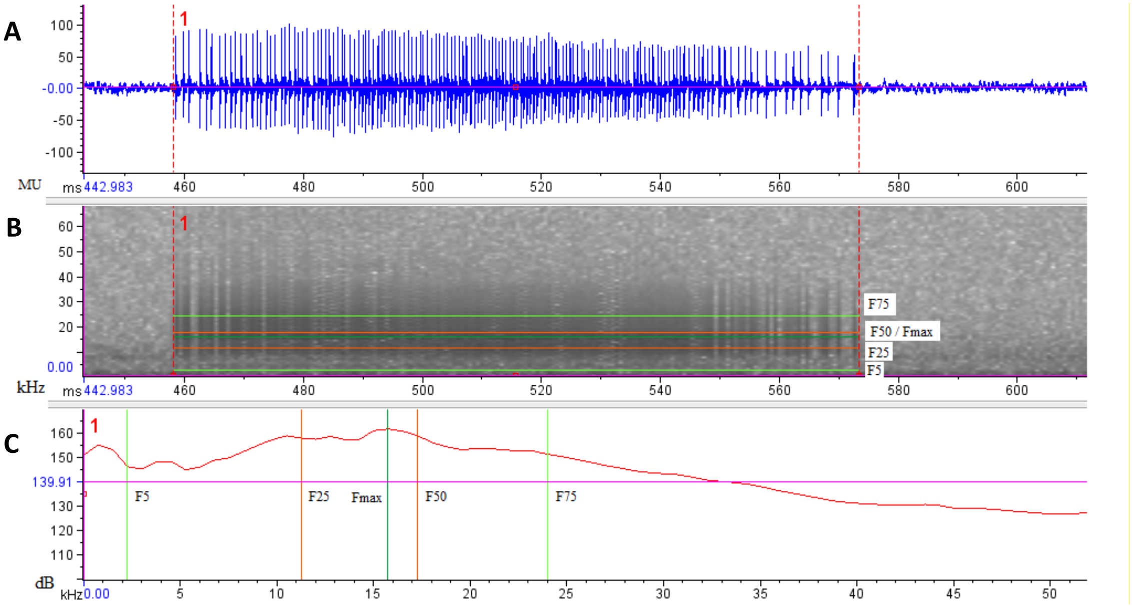


Figure 3. Oscillogram and spectrogram and Power spectrum of the species *Odontomachus laticeps*, **A** Oscillogram with a chirp, **B** Spectrogram with the magnification of the first signal showing the dominant frequency (Fmax) frequencies at (F5) 5% (F25) 25% (F50) 50% and (F75) 75% of the signal energy, **C** Power spectrum of the selected signal showing the maximum frequency (Fmax) and frequencies at (F5) 5% (F25) 25% (F50) 50% and (F75) 75% of the signal energy. Spectrogram parameters: Window type: Hann, Window size: 128, Overlap: 50%.

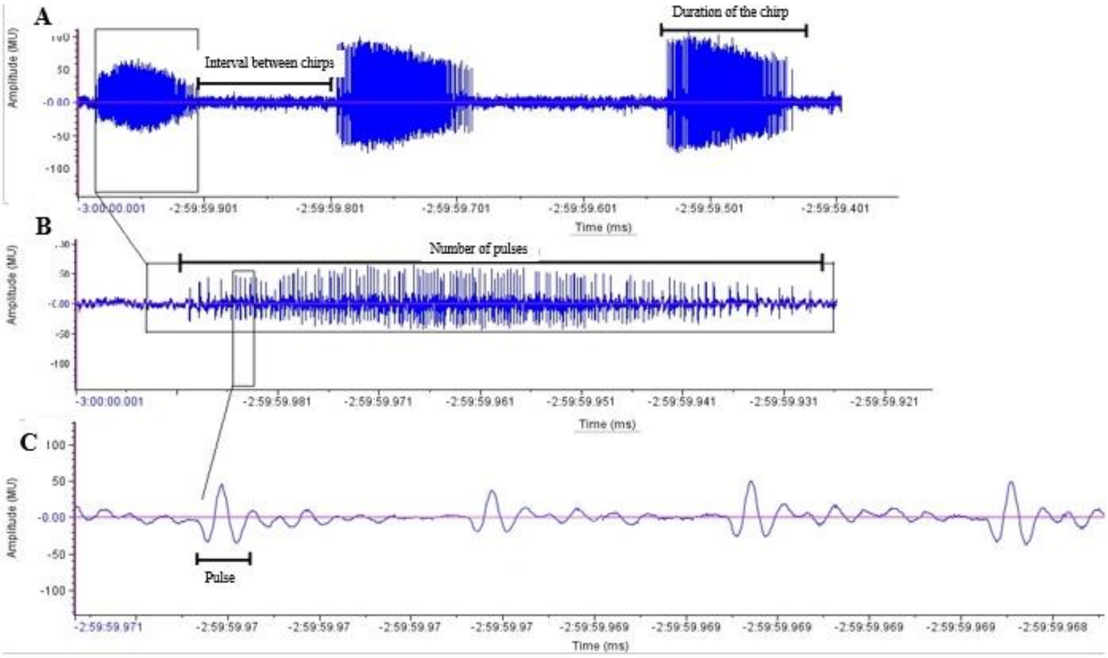


Figure 4. Oscillogram showing the acoustic parameters extracted from the stridulatory signals of *Odontomachus laticeps*, **A** oscillogram with a sequence of three chirps showing the duration of the chirp and the interval between chirps, **B** oscillogram with the chirp showing the number of pulses, **C** oscillogram with magnification of one pulse of the chirp.

Table 1. Spectro-temporal parameters of the acoustic signals recorded from ants and their description are taken from Solfware Raven Pro 1.6.

Acoustic parameter	Description
Bandwidth	Difference between the highest and lowest frequency within the selection.
Lower frequency	The lowest frequency within the selection.
Higher frequency	The highest frequency within the selection.
Frequency 5%	Frequency that divides the selection into two frequency intervals containing 5% and 95% of the energy.
Frequency 95%	Frequency that divides the selection into two frequency intervals containing 95% and 5% of the power.
Peak frequency	The frequency at which the maximum power/peak frequency occurs within the selection.

1st frequency quartile	Time point that divides the selection into two intervals containing 25% and 75% of the energy within the selection.
Duration of chirp	Duration of the signal within the selection.
Interval between chirps	Duration between the emission of one signal and the next.
Average chirp interval	Selection of 5 chirp interval values divided by 5
Number of pulses	Number of pulses in each signal emitted.

Morphological characteristics of ants

To evaluate how morphological characteristics influence the spectro-temporal patterns of acoustic signals (objective 2), we collected up to six subordinate and six dominant workers from each bait. We captured workers of the same species with visually similar sizes and of the worker and soldier castes when present. The captured workers were individually recorded in the laboratory in the context of a distress call and identified to genus using an ant species identification guide (Baccaro et al. 2015), and later to species using specific articles and by comparison with the collection deposited at UFAM.

Scanning electron microscopy was used to visualize the stridulatory apparatus and the trichoid sensilla, following the protocol of (Barsagade et al. 2013). In the laboratory of the Multiuser Center for the Analysis of Biomedical Phenomena at the Federal University of Amazonas (CMABio), the ants were cleaned with 70% alcohol, followed by dissection using a stereoscopic microscope, where the antennae with the cephalic region were removed, as well as the region of the stridulatory apparatus between the post-peciole and the gastrula in Myrmicinae and in the first segment of the gastrula in Ponerinae. The prepared individuals were fixed in carbon tape, plated in 8 nm gold-palladium with a Jeol Smart Coater and photographed with a Jeol JSM IT500HR Scanning Electron Microscope (SEM). We used the TPSDig 264 software (version 2.32) to measure the following morphometric variables according to (Ferreira et al. 2010). Four morphological characteristics of the stridulatory apparatus were measured Figure 5: length of the pars-stridens (mm), width of the pars-stridens (mm), number of striations on the pars-stridens at 10 µm and average distance between the ridges and photographs of the tip of the antenna of the ants to observe the presence of curved trichoid sensilla (Ferreira et al. 2010).

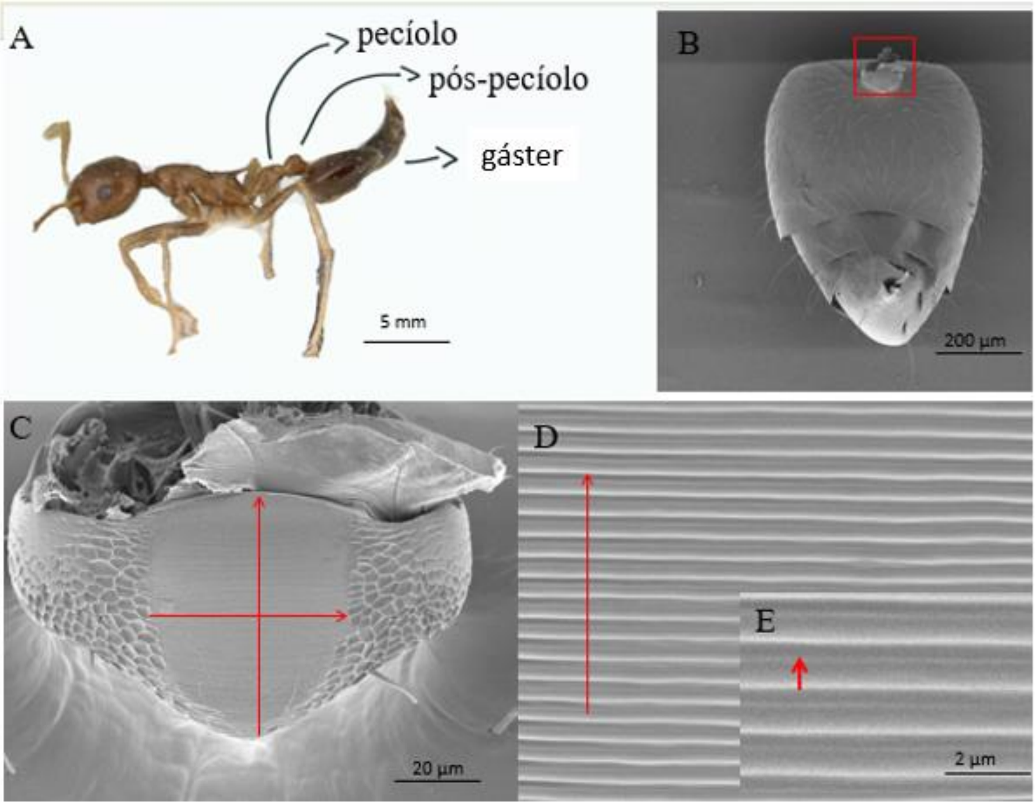


Figure 5. Structures of the stridulatory apparatus of the dominant species *Crematogaster tenuicula*: **A** position of the stridulatory apparatus, **B** location of the stridulatory apparatus in the post-peciole region, B height and width of pars stridens (mm), **C** number of ridges in 10 µm, **D** distance between pars-stridens.

DATA ANALYSIS

To control for the effect of the different number of dominant and subordinate workers, we divided the total number of acoustic signals by the number of workers of each species present in the baits (frequency of acoustic signals per worker). We used the frequency of acoustic signals per worker as the dependent variable and the species classification (dominant or submissive) as the independent variable in an Analysis of Variance (ANOVA). In this analysis, the sample unit was the bait. It was not possible to use paired ANOVA, because in some cases only dominant or subordinate species were present in the baits. The adequacy of the model was investigated using residual analysis plots.

We summarized the spectro-temporal parameters of the ants' acoustic signals using a PCA (Principal Component Analysis). We created two PCAs, one using only spectral data of the acoustic signals and another PCA using only temporal parameters of the acoustic signals. We used the average of the spectro-temporal parameters of the acoustic signals of each ant species per bait. The PCA summarizes and concentrates the information from the attributes (spectro-temporal parameters, in our case) into ordination axes. In this analysis, the sample unit was the average of each parameter per species per bait. We used the first two axes of the PCA as dependent variables in an Analysis of Variance Multivariate by Permutation (ADONIS) and the classification of the species (dominant or submissive) as a predictor variable. This analysis compares the position, i.e. whether the values of the acoustic signals are different between the dominant and subordinate species. However, in some cases, this analysis can confuse position with dispersion of values. We therefore used a dispersion analysis between groups (Multivariate Homogeneity of Groups Dispersions - BETADISPER) to better investigate possible differences between the acoustic signals of dominant and subordinate species. In both analyses, the observed results were compared with 999 permutations.

We used analysis of covariance (ANCOVA) to investigate the relationship between the number of pulses of the acoustic signals recorded in the context of distras-calls and the height of the stridulatory apparatus, as a function of the classification of the species as dominant or submissive. We also used an ANCOVA to investigate the relationship between the mean interval between pulses and the distance between ridges of the stridulatory apparatus as a function of the classification of the species as dominant or subordinate. All morphological measurements were divided by the length of the ant (Weber length), making the morphological measurements standardized by the size of the ant. We used residual analyses to investigate the adequacy of the models. All analyses were carried out in the R program (R Core Team 2023).

RESULTS

In total of 93 baits were installed and monitored, resulting in 186 hours of observation in the field. A total of 370 bait recordings and 110 individual laboratory recordings were made. We collected 18 species of ants belonging to the genera *Blepharidatta*, *Crematogaster*, *Pheidole*, *Solenopsis*, *Ochetomyrmex*, *Wasmannia*, which were classified as dominant species, and 8 species belonging to the genera *Atta*, *Camponotus*, *Dolichoderus*, *Ectatomma*, *Neoponera* and *Mycetomoellerius* classified as subordinate.

We observed that the dominant species emitted acoustic signals when arriving at the bait, and when the number of individuals of a species increased on the bait, i.e. when recruitment behavior occurred, the emission of acoustic signals became constant in some species belonging to the genera *Solenopsis* and *Pheidole*. These species use the mass-recruitment strategy, often resulting in mass recruitment with the presence of soldiers. In contrast, species from the genera *Blepharidatta*, *Crematogaster* and *Ochetomyrmex* emitted acoustic signals in a non-constant manner. Species from these genera normally use the group-recruitment strategy, recruiting more individuals in groups, not always with the presence of soldiers. We observed events of aggression, such as biting or the deposition of pheromones, between workers of different species.

Atta sexdens and *Mycetomoellerius farinosus* were the only species of the Myrmicinae subfamily classified as submissive (possibly the bait was located on the foraging trail). The subordinate species *Ectatomma brunneum, E. lugens* and *E. edentatum* and *Neoponera apicalis*, which were almost always the first to arrive at the bait, used the strategy of solitary recruitment or tandem running, where one or two individuals took a bit of bait and returned to the nest and then back to the bait, performing lurking behaviors by standing near the bait. For these species, the presence of dominant species such as *Pheidole bioconstricta* caused aggression events accompanied by short acoustic emissions. Other species such as *Camponotus sp*. *Camponotus novogranadensis*, *Nylanderia sp*. *Nylanderia sp.1*, *Nylanderia sp.10*, *Nylanderia “amarela”* and *Dolichoderus sp*. arrived at the bait in a wandering manner, did not emit acoustic signals and did not recruit nest mates and were often attacked by dominant species, except for *Camponotus novogranadensis* which emitted percussion signals.

Dominance behavior and emission of acoustic signals

The average frequency of acoustic signals emitted by ants during dominance behavior was higher among subordinate ant species compared to dominant species (ANOVA; $p = 0.045$, $N = 93$). On average we recorded 6.5 acoustic signals (SD = 4.5) among subordinate species, and only 2 acoustic signals (SD = 2.15) per dominant species worker (Figure 6).

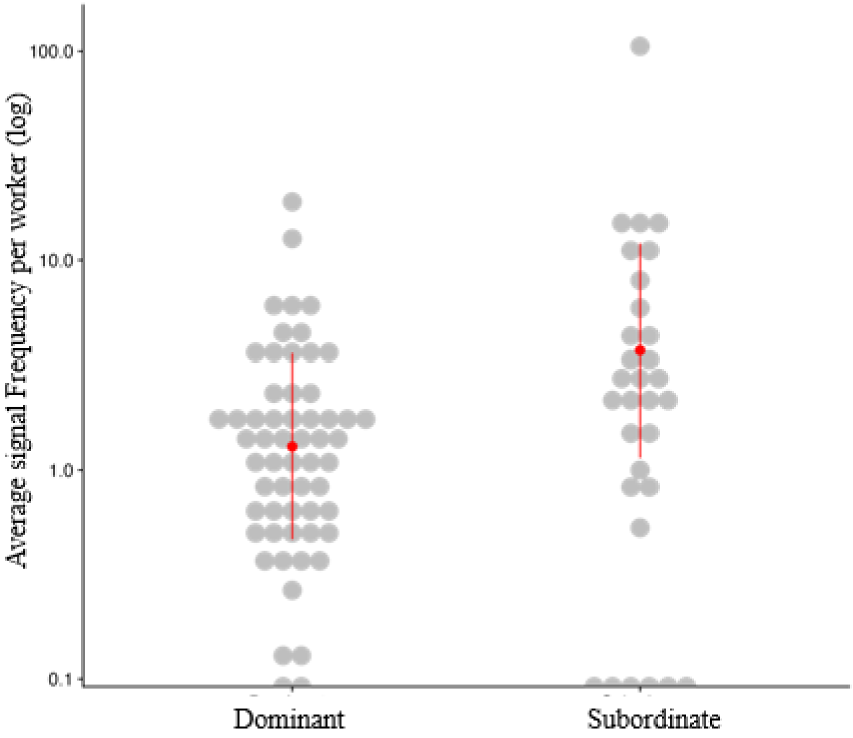


Figure 6. Average frequency of acoustic signals emitted by workers during baiting.

The two axes of the ordination of the spectral parameters of the acoustic signals (PCA) captured ~80% of the total variation in the data. Multivariate permutation analysis indicated that there are no differences in spectro-temporal parameters between dominant and subordinate species (ADONIS; R2 = 0.0545, F1,51 = 2.953, P = 0.065). Figure 5, however, clearly shows that spectral parameters of dominant ants are actually a subset of the spectral parameters of subordinate ants (BETADISPER; F1,51 = 23.88, P < 0.001).

The results, looking only at the temporal parameters of the acoustic signals, were slightly different. The two axes of the ordination using temporal parameters of the acoustic signals (PCA) captured ~85% of the total variation in the data. The combination of temporal parameters was very similar between dominant and subordinate ant species (Figure 7), both in position (ADONIS; R2 = 0.017, F1,51 = 0.900, P = 0.435) and dispersal (BETADISPER; F1,51 = 1.849, P = 0.195).

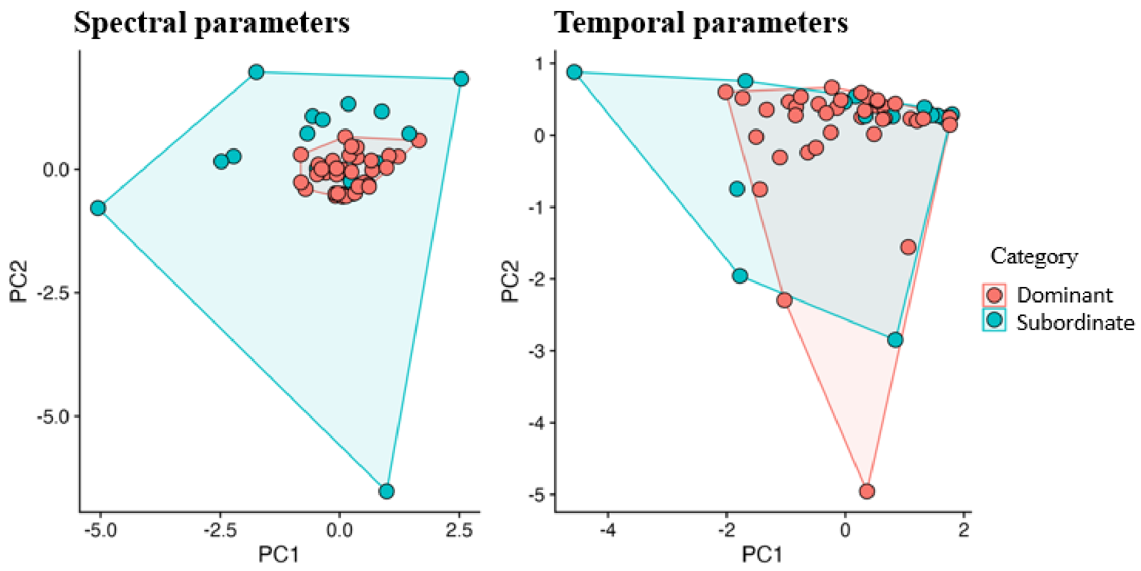


Figure 7. Ordering of the spectro-temporal acoustic parameters emitted by ants during bait stinging.

Morphology involved in the emission and reception of acoustic signals

The stridulatory apparatus of the dominant ant species varied in shape from rounded (Figure 11, A) to square (Figure 10 A). We reported bifurcated ridges for the first time in the species *Wasmannia auropunctata* (Figure 12, G). We also noted the presence of basicoid sensilla in the medial region positioned to the right and left of the stridulatory apparatus in red (Figure 10, C), as well as the presence of pillars in most species of *Pheidole* (Figure 11, G) and in *Crematogaster* (Figure 10, C). We also observed the presence of these structures in the stridulatory apparatus of subordinate ants (Figure 13).

The subordinate ant species had curved trochoid sensilla in the medial region, positioned to the right and left of the stridulatory apparatus (Figure 14). *Atta sexdens* had a stridulatory apparatus in the region of the post-peciole and gastrula like the other Myrmicinae, ants of the genera *Camponotus*, *Dolichoderus* did not have a stridulatory apparatus.

We found curved trichoid sensilla with different morphologies ranging from curved and rounded at the base to open structures in the basal or apical region located in the anterior apical region of the antennae of dominant ants from the genera *Blepharidatta*, *Crematogaster*, *Pheidole*, *Solenopsis* and *Ochetomyrmex* (Figure 15). There were fewer sensilla in the ventral region of the antenna. In subordinate ants of the genera *Ectatomma*, *Neoponera* and *Atta*, we found more curved trichoid sensilla on the antennae and fewer in the genus *Camponotus* (Figure 16).

Stridulatory apparatus morphology and temporal parameters

We measured and observed the presence of the stridulatory apparatus and mechanoreceptor sensilla in 291 individuals of 51 ant species collected during the presence in dominance experiment and recorded the acoustic signals in the context of distress-calls in the laboratory of each individual. We found a relationship between the temporal acoustic parameter (number of pulses) and the morphological variable (height of the stridulatory apparatus) (R2 = 0.37, F2,20 = 7.473, p = 0.003). In these models the two variables were significant, showing a positive relationship between the number of pulses and the height of the stridulatory apparatus (b = 0.650, P = 0.001), and that the dominant species emitted more pulses than the subordinate species (b = -0.329, p = 0.034). However, subordinate species showed greater variation in the size of the stridulatory apparatus (Figure 8).

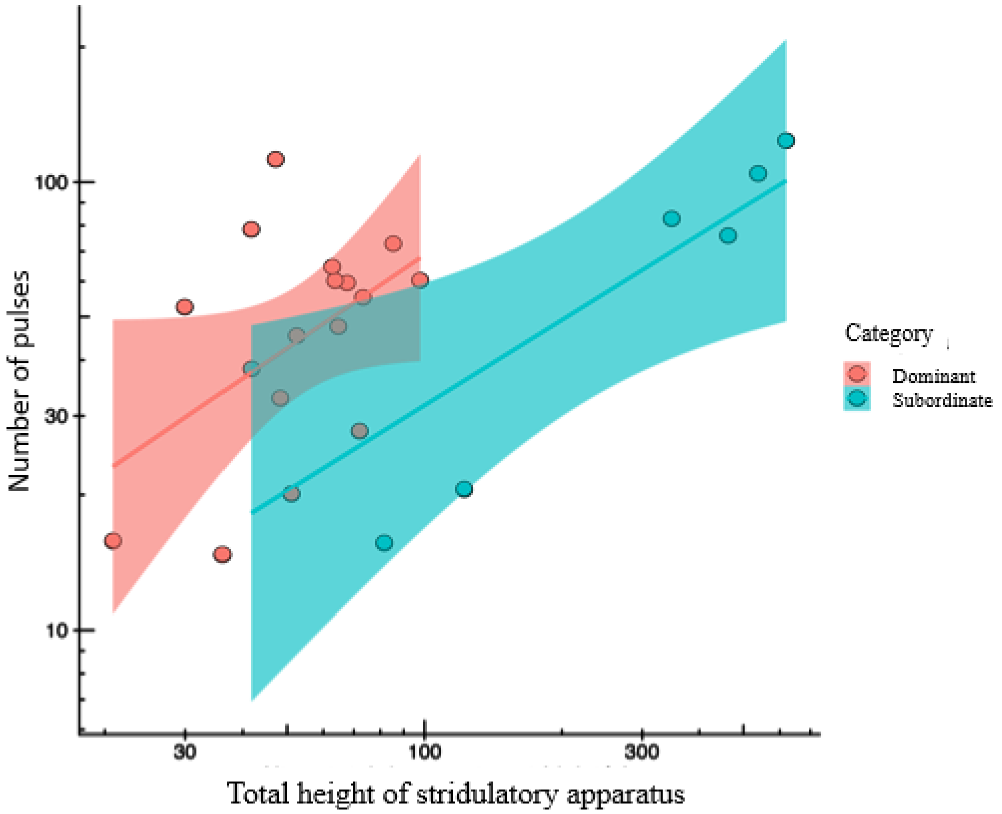


Figure 8. Relationship between number of pulses and total height of stridulatory apparatus in dominant and subordinate species.

We found no relationship between the average interval between pulses and the distance between ridges of the stridulatory apparatus (R2 = 0.043, F2,20 = 1.495, p = 0.248). The interval between pulses was also similar between dominant and subordinate ants (Figure 9).

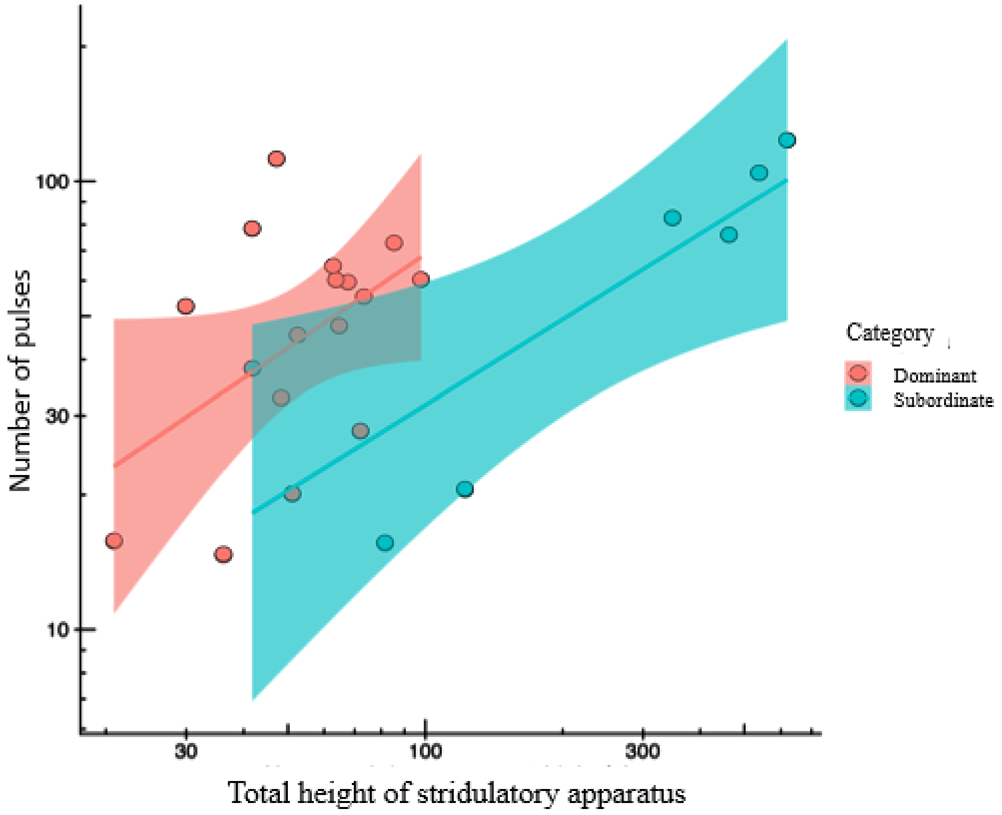


Figure 9. Relationship between average pulse interval and distance between ridges in dominant and subordinate species

DISCUSSION

The emission of acoustic signals by ants during recruitment events is notorious, however, records are lacking during interactions between species during the exploitation of food resources in the wild (Hölldobler 1999; Masoni 2021; Roces 2022). In this article, we observed and recorded the acoustic signals of recruiting ants during dominance behavior for food resources and our results suggest that subordinate species emit on average more acoustic signals per worker than dominant species. Dominant ants had different spectral parameters to subordinate ants, but their temporal parameters were similar. In addition, we analyzed the relationships between the morphology of the stridulatory apparatus and the temporal parameters of the acoustic signals of distress-calls of dominant and subordinate ants. Our results suggest a positive correlation between the height of the stridulatory apparatus and the number of pulses in the acoustic signals. However, we observed no correlation between the distance between the ridges and the average pulse interval. Finally, we observed the presence of curved trichoid sensilla on the tips of the antennae of dominant and subordinate ants. Future studies could evaluate other contexts of ant behavior as well as other morphological and spectro-temporal variables of their acoustic signals.

Numerical dominance and spectro-temporal parameters

Subordinate ants such as those of the genera *Ectatomma* and *Atta* had a higher frequency of acoustic signals emitted by workers compared to dominant species. This result contradicted our hypothesis. Perhaps acoustic signals are emitted more frequently by subordinates because they are outnumbered and need to defend themselves against attacks from dominant ants. One piece of evidence that supports this conclusion is that acoustic signals in Ponerinaes, the majority of subordinate species in this study, are normally emitted in distress call contexts (Ferreira 2014). However, the signals recorded during foraging were not as long and constant as during distress-calls, but instead were short, shorter and rapid during the presence of or aggression towards the bait. Anthropomorphically, we can interpret these emissions as "shouting to scare off a pack of dogs", i.e. in the context of defense, the subordinate ants defend themselves with aggression and acoustic emissions, which can make it easier for the individual to escape with the bait. This does not mean that the emission of acoustic signals by dominant ants is not important for the recruitment of other nest workers, since the emission of acoustic signals over the bait can maximize the marking of pheromone trails, increasing recruitment at close range (Hickling and Brown 2000).

This research evaluated for the first time the relationship between the spectro-temporal parameters that make up acoustic signals and the dominance behavior of ants. The spectral parameters, i.e. the frequency values in Hz, were different between dominant and subordinate species. The spectral parameters of dominant species are a subset of the spectral parameters of subordinate species. This makes sense, given that Myrmicinae ants emit acoustic signals mostly composed of low frequencies (Hickeling and Brown 2000) and subordinate ants such as Ponerinaes can emit acoustic signals with higher frequency values (Esperson 1994). With this evidence we can reconsider that the acoustic signals in Poneromorphs are not a simple generalist signal as suggested by Markl (1973). Our results suggest that the acoustic signals emitted by subordinate ants may carry more information than an indication of stress or discomfort. Spectral values can help modulate dominance strategies between ant species.

Unlike the spectral parameters, the temporal parameters showed no differences between the behavioral categories. This result contradicts previous results based on distress call signals (Ferreira 2014), in which temporal parameters were good predictors of species identity. Here we recorded acoustic signals in another behavioral context during competition for resources, mainly during rapid events of aggression over the bait. In addition, we also show another facet of the use of acoustic signals in Poneromorphs, since detailed studies of acoustic characteristics are scarce and more focused on distress call sounds (Ferreira 2009; Silveira 2015; Golden and Hill 2019;). Therefore, it would not be advantageous for subordinate ants to spend more time on the bait emitting long acoustic signals, such as when held by tweezers (a protocol that simulates predation), which could lead to attacks by dominant ants. However, the subordinate species emitted short signals and quickly withdrew from the bait.

There are some reports of interactions mediated by acoustic signals with other groups. For example, the beetle *Paussus favieri* has acoustic parameters very similar to those of the ant *Pheidole pallidula*, which it parasitizes (Fattorini et al. 2021). Ants can even shape hierarchical strategies in the care preference of nymphs within the colony, mediated by acoustic signals as in the *Myrmica scabrinodis* species (Casacci 2013). Our results expand the repertoire of the use of acoustic signals by ants, demonstrating the emission of acoustic signals during foraging behavior in ants. Given the repertoire and variability of the signals, our results also suggest that there is some complexity in communication mediated by acoustic signals in ants. This new evidence may help provide a broader view of the evolution of acoustic communication within one of the most diverse and abundant groups on the planet.

Morphology and distress-calls

Unlike the acoustic signals recorded in the context of recruitment, we also recorded the distress-call acoustic signals of dominant and subordinate ants. Distress-call signals are emitted during the immobilization of some region of the ant's body, simulating an attempt at predation. Studies use this acoustic signal and the morphology of the stridulatory apparatus to identify cryptic ants, clarifying relationships between species belonging mostly to the Ponerinae subfamily (Pavan et al. 1997; Ferreira 2010; Peña Carrillo 2022).

We have found diversity in the sounds or acoustic signals of distress-calls emitted by Myrmicinae ants, most of which are composed of two signal subunits, i.e. disyllabic signals. This structure can be moved in both directions and seems to be modulated by the stimulus that the ant can receive (Masoni 2021). Just as we observed disyllabic signals in Ponerinae ant species, we also observed that the temporal parameter number of pulses is correlated with the height of the stridulatory apparatus. Dominant species emitted more pulses than subordinate species, with subordinate species having greater variation in the size of the stridulatory apparatus. The number of pulses is related to the duration of the signal, the greater the number of pulses, the longer the duration of the acoustic signal. However, we did not observe a relationship between the average interval between pulses and the distance between the crests, which may be involved in the mechanics of the movement of the acoustic signals (Yao et al. 2018). This is the first time that a study has shown a relationship between the morphology of the stridulatory apparatus and the acoustic parameter (number of pulses). Further analysis of this structure and its relationship with spectral parameters is still needed.

We observed the presence of sensilla in the lower third, to the right and left of the stridulatory apparatus, corroborating previous studies (Grasso et al. 1998; Álvarez 2009; Castro 2015). Research suggests that these sensilla have a proprioceptive function, i.e. they generate information about the position of the stridulatory apparatus for the individual. These sensilla are found in dominant Myrmicinae and subordinate Poneromorph ants and are not exposed, being located close to the vibration-generating mechanism protected by a post-peciole envelope. Further studies should consider the presence and function of these structures, which seem to have a mechanoreceptive function in receiving vibrations from the substrate. We also observed the presence of pillars, specific cuticular formations under the *pars stridens* or stridulatory apparatus, which support that this structure may have a signal amplifying function (Álvarez 2009; Castro 2015).

Acoustic signals are received via the substrate through the cord organs and possibly through the air in the near field via mechanoreceptor sensilla (Roces and Tautz 2001;

Hickling and Brow 2000). We did not evaluate the relationship between sensilla morphology and spectro-temporal parameters. However, we bring new information about the presence and morphological characteristics of curved trichoid sensilla in several species of dominant ants, with great morphological variety. Subordinate ants belonging to the Ponerinae subfamily showed a greater number, but less variety, of curved trichoid sensilla. Finally, we hope to contribute to the knowledge of this structure, still little explored within the group, which may be involved in acoustic reception through the air in ants.

In general, our results suggest that on average, subordinate ants such as Ponerinae during agonistic interactions emit more acoustic signals than dominant Myrmicinae ants. And the spectral parameters of dominant ants are different from the spectral parameters of subordinate ants, but the temporal parameters are similar between the groups, meaning that the spectral values of acoustic signals can help modulate dominance strategies between ant species. Regarding the morphology of the stridulatory apparatus, the characteristic height of the stridulatory apparatus was correlated with the number of pulses in the signal, but the average pulse interval was not related to the distance between the ridges. We showed new morphologies of curved trichoid sensilla present in the groups. Thus, we suggest that there is complexity in communication measured via acoustic signals in ant species, and we hope to contribute to future studies on acoustic behavior and the evolution of this communication route for the Formicidae group.

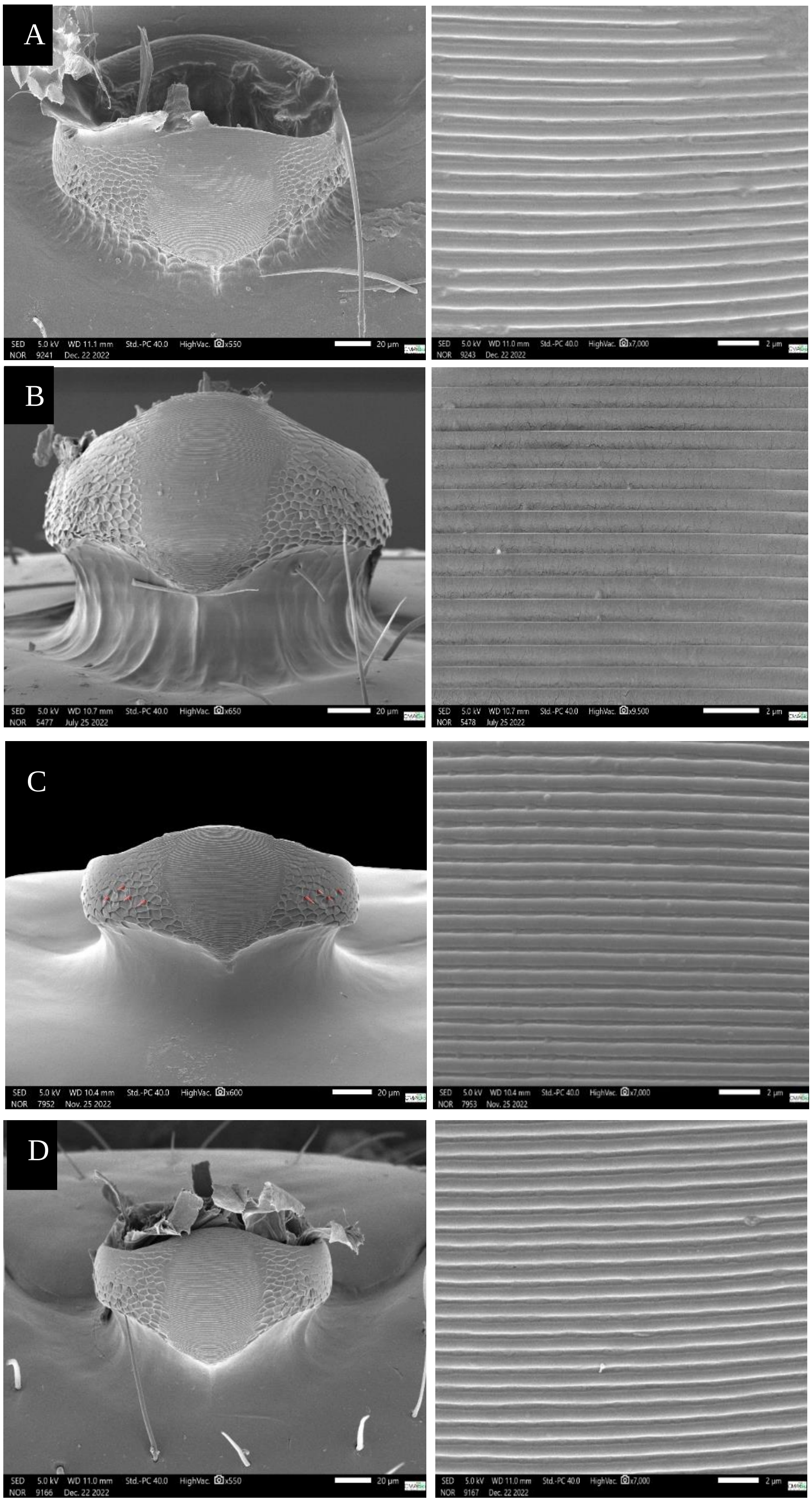
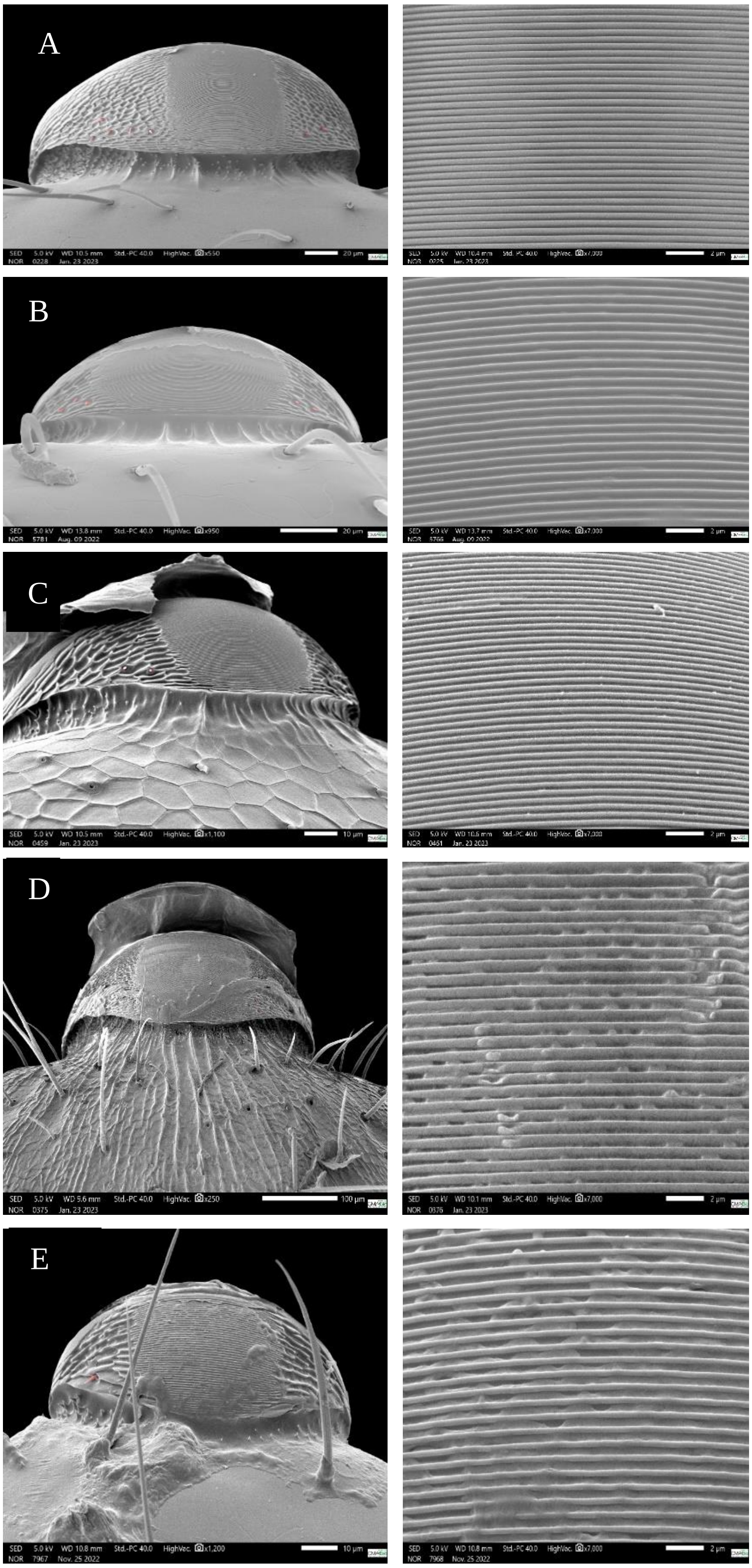


Figure 10. Scanning electron microscopy (SEM) images of the stridulatory apparatus between 550 X and 700 X Zoom and ridges at 7,000 X in dominant ant species belonging to the Subfamily Myrmicinae of the genus *Crematogaster*: ***A*** *Crematogaster acuata*, ***B*** *Crematogaster brasiliensis*, **C** *Crematogaster longispina*, **D** *Crematogaster tenuicula*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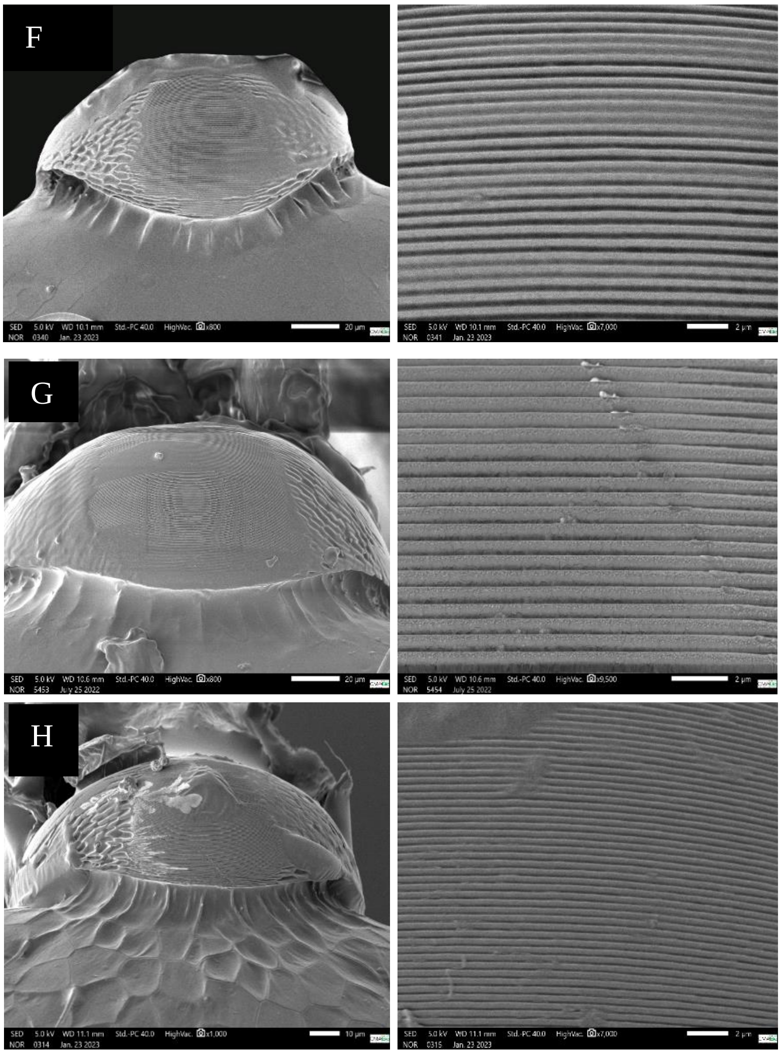

Figure 11. Scanning electron microscopy (SEM) images of the stridulatory apparatus between 550 X and 700 X Zoom and ridges at 7,000 X in dominant ant species belonging to the Subfamily Myrmicinae of the genus *Pheidole*: **A** *Pheidole sp. 60* soldier, **B** *Pheidole sp. 60* worker, **C** *Pheidole* sp. *25* worker **D** *Pheidole* sp. *30* soldier, **E** Pheidole sp.18 , **F** *Pheidole meinerte* yellow soldier**,** **G** *Pheidole meinerte* yellow worker**,** **H** *Pheidole cephalica***,** **I** *Pheidole deima* soldier**,** **J** *Pheidole* sp. 15 soldier

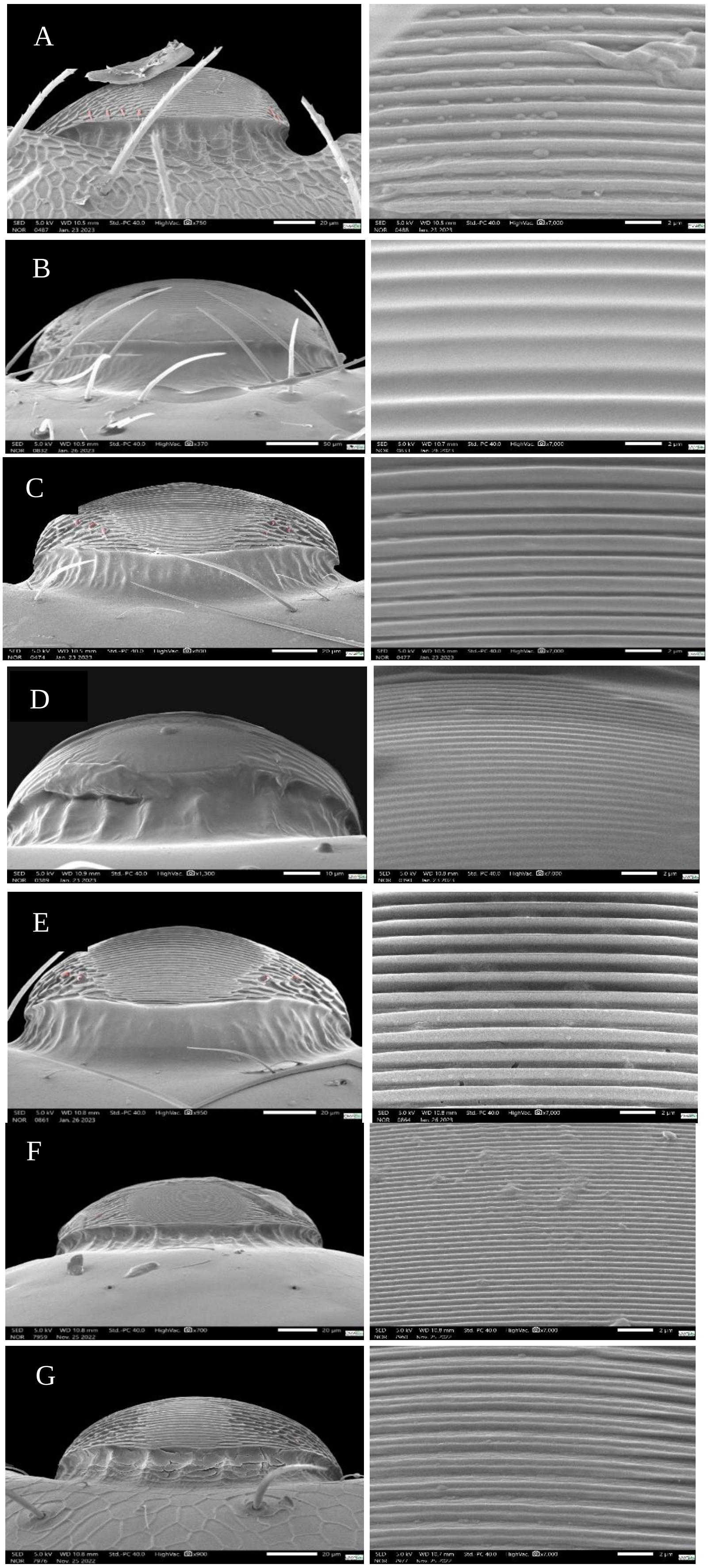

Figure 12. Scanning electron microscopy (SEM) images of the stridulatory apparatus between 550 X and 700 X Zoom and crests at 7,000 X in dominant ant species belonging

to the Subfamily Myrmicinae of the genera: *Blepharidatta*, *Solenopsis*, *Ochetomyrmex* and *Wasmannia*, **A** *Blepharidatta brasiliensis*, **B** *Solenopsis gr. geminata* soldier, **C** *Solenopsis gr. geminata* worker, **D** *Solenopsis* sp. 1, **E** *Solenopsis* sp. 2, **F** *Ochetomyrmex semipolitus,* **G** *Wasmannia auropunctata*

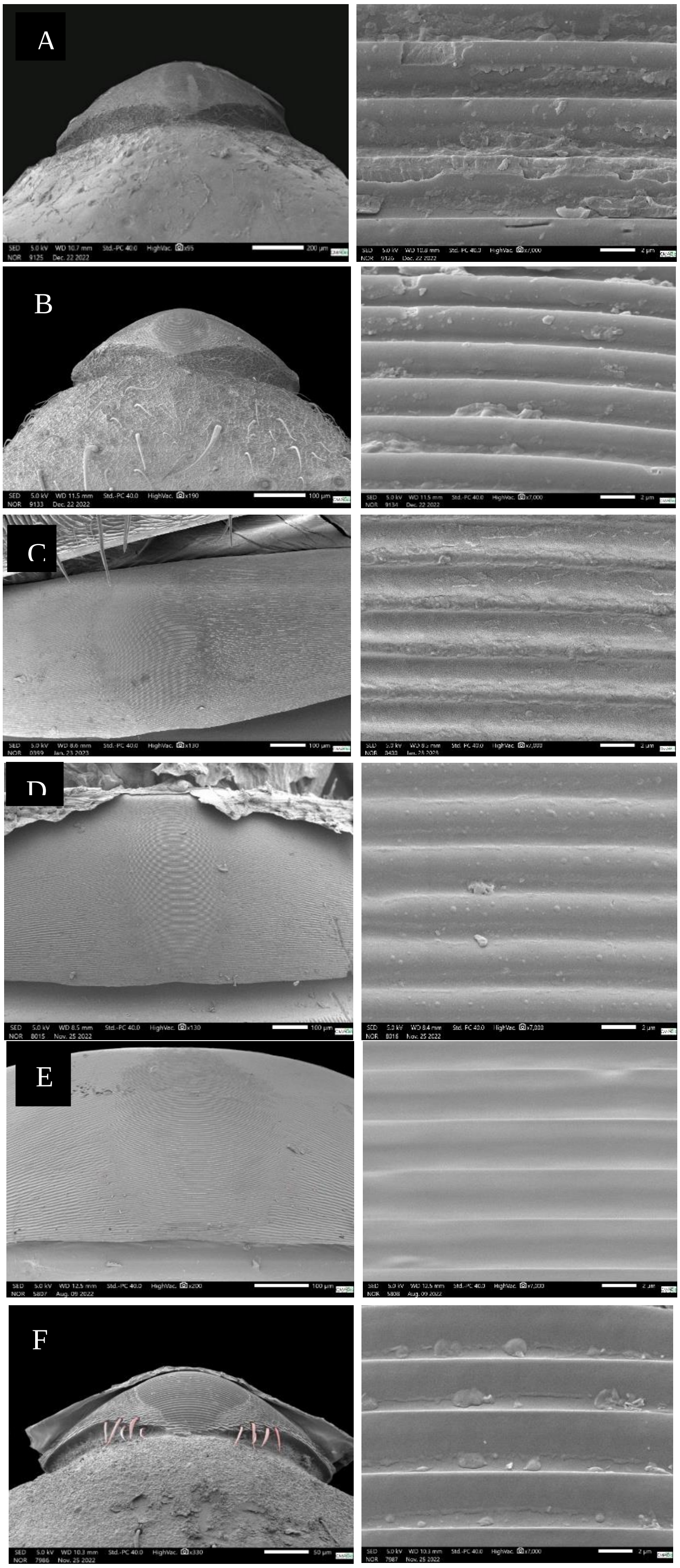

Figure 13. Scanning electron microscopy (SEM) images of the morphology of the stridulatory apparatus in subordinate ant species of the genera *Atta, Ectatomma, Neoponera, Mycetomoellerius*: **A** *Atta sexdens* soldier, **B** *Atta sexdens* worker, **C**

Ectatomma brunneum, **D** *Ectatomma lugens*, **E** *Neoponera apicalis*, **F** *Mycetomoellerius pharinosos*

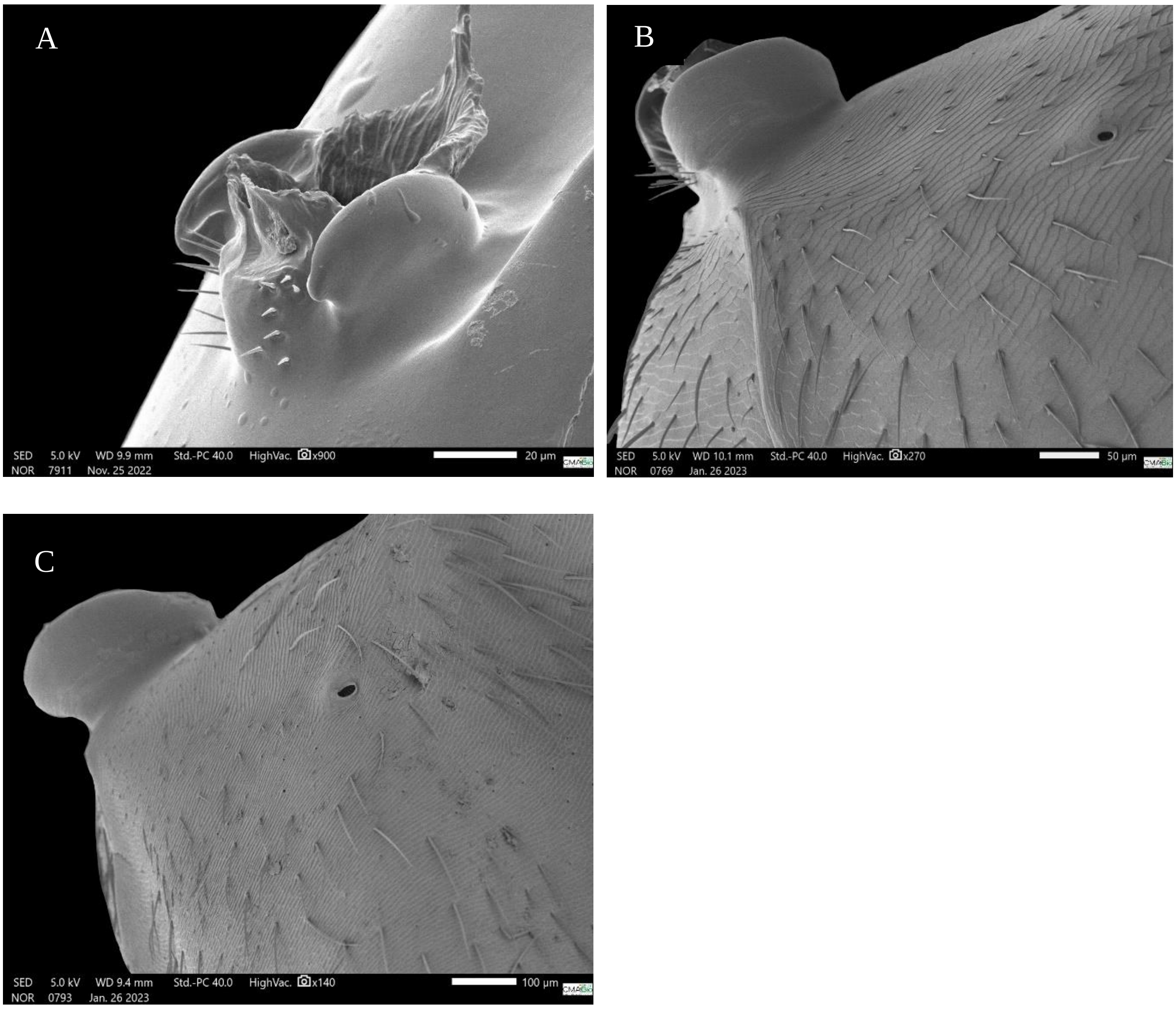

Figure 14. Scanning electron microscopy (SEM) images of subordinate ant species that did not have a stridulatory apparatus **A** *Nylanderia sp*. **B** *Camponotus femoratus*, **C** *Camponotus rapax*

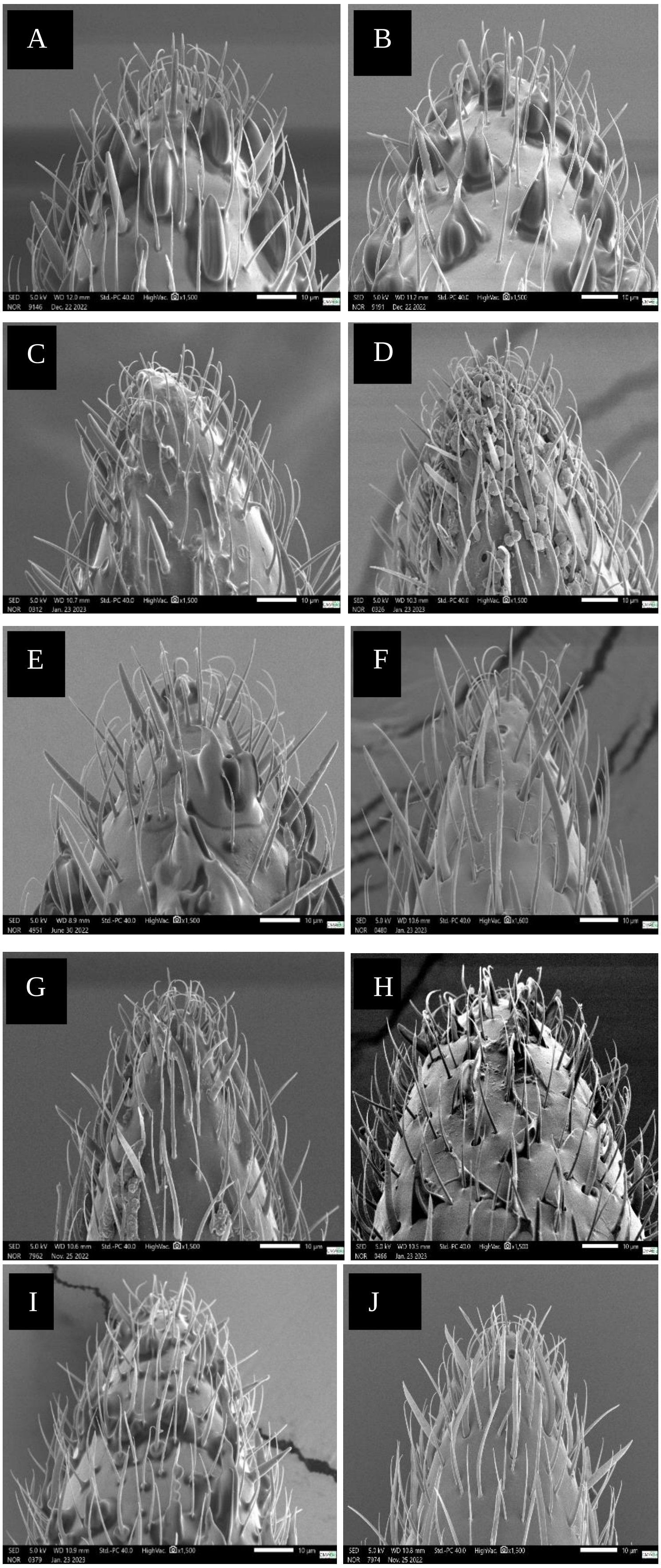
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Figure 15. Scanning electron microscopy (SEM) images of the curved trichoid sensilla in the apical region of the antenna of the main dominant ant species at 1.200 X: **A** *Crematogaster acuata*, **B** *Crematogaster brasiliensis*, **C** *Pheidole cephalica*, **D** *Pheidole deima*, **E** *Pheidole meinerte amarela*, **F** *Blepharidatta brasiliensis*, **G** *Ochetomyrmex semipolitus*, **H** *Solenopsis gr. geminata*, **I** *Solenopsis sp. 1*, **J** *Wasmannia auropunctata*

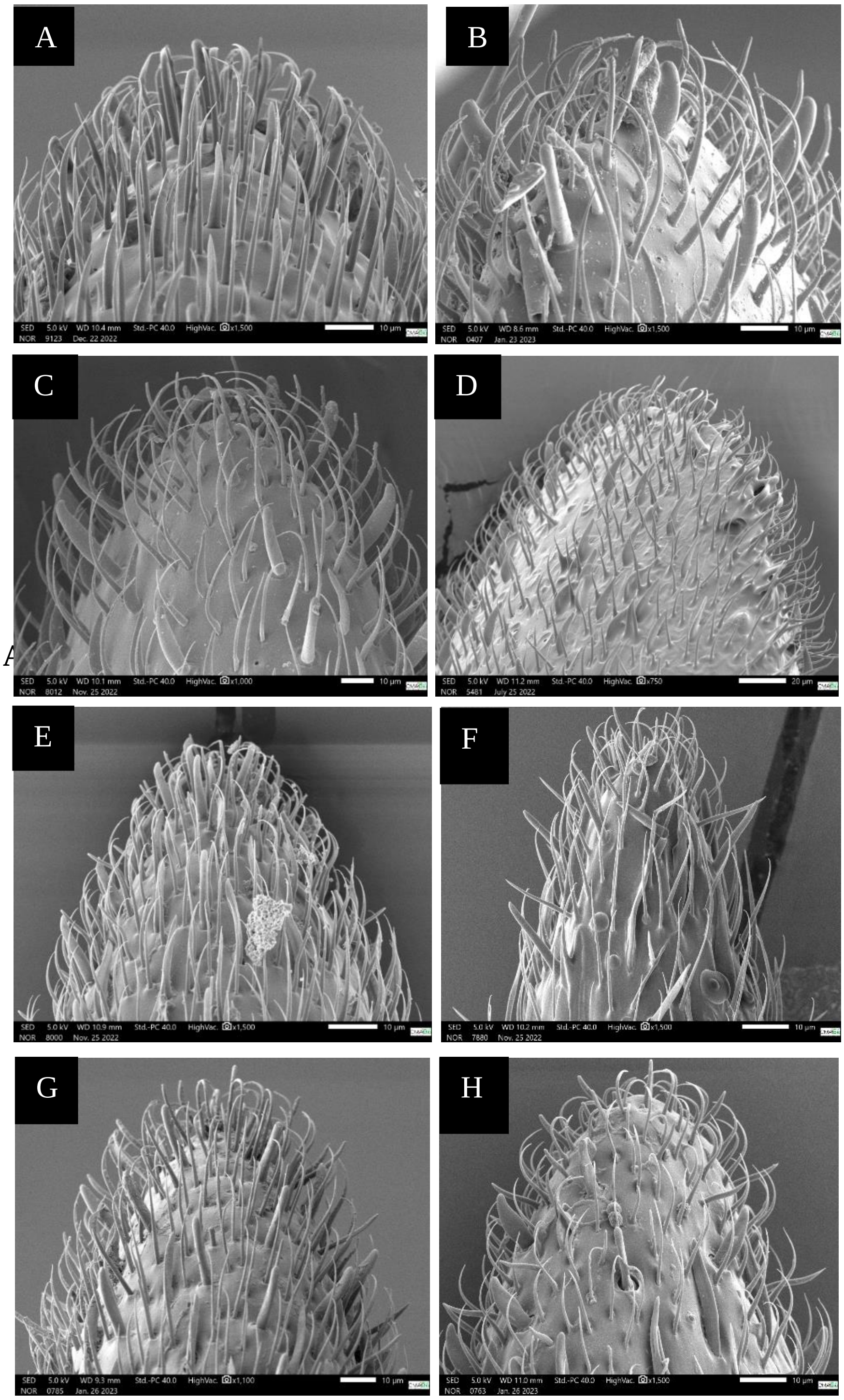

Figure 16. Scanning electron microscopy (SEM) images of the curved trichoid sensilla in the apical region of the antenna of the main subordinate ant species at 1.200 X: **A** *Atta sexdens* soldada, **B** *Ectatomma brunneum*, **C** *Ectatomma lugens*, **D** *Neoponera apicalis*,

E *Mycetomoellerius farinosus,* **F** *Nylanderia sp.* **G** *Camponotus rapax*, **H** *Camponotus femoratus*

FINAL CONSIDERATIONS

In this research, we proposed an innovative protocol for collecting acoustic signals emitted by ants, which facilitated the observation of various behaviors in the natural environment. We also tested the effectiveness of these signals for species diagnosis, as well as exploring how numerical dominance behavior and morphological characteristics influence the emission and spectro-temporal parameters of acoustic signals in soil ant species in the Central Amazon.

In the first chapter, we recommend the use of microphones and recorders with integrated amplifiers to capture low-amplitude signals, ensuring the detection of a wide range of frequencies emitted by the ants. We also suggest the combined use of spectral and temporal parameters in the analyses, with temporal parameters proving to be the best predictors for species delimitation based on acoustic signals. Our results indicate that the use of these devices, combined with a simplified recording protocol, can provide valuable insights for behavioral, taxonomic and evolutionary studies.

In the second chapter, we verified that dominance behavior among ants is mediated by acoustic signals, especially those emitted during recruitment or in defense contexts after episodes of aggression between species. Our results show that subordinate ants, such as those in the Ponerinae subfamily, emit more acoustic signals than dominant ants, such as the Myrmicinae. While the spectral parameters of the signals differ between dominants and subordinates, the temporal parameters are similar. This suggests that spectral values may play an important role in modulating dominance strategies between different species. In addition, we identified a correlation between the height of the stridulatory apparatus and the number of pulses emitted in the signal, although the average interval between pulses showed no relationship with the distance between the stridulatory crests. We also described new morphologies of curved trichoid sensilla, associated with hierarchical context in the use of resources.

Thus, our results suggest a complex acoustic communication between ant species, highlighting the importance of acoustic signals in structuring social interactions in the

Formicidae group. We hope that this study will contribute to the advancement of research into acoustic behavior and the evolution of this communication pathway within the group.

ACKNOWLEDGEMENT

The authors thank FAPEAM for funding the master's scholarship and CEBAM for their support and field logistics.

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