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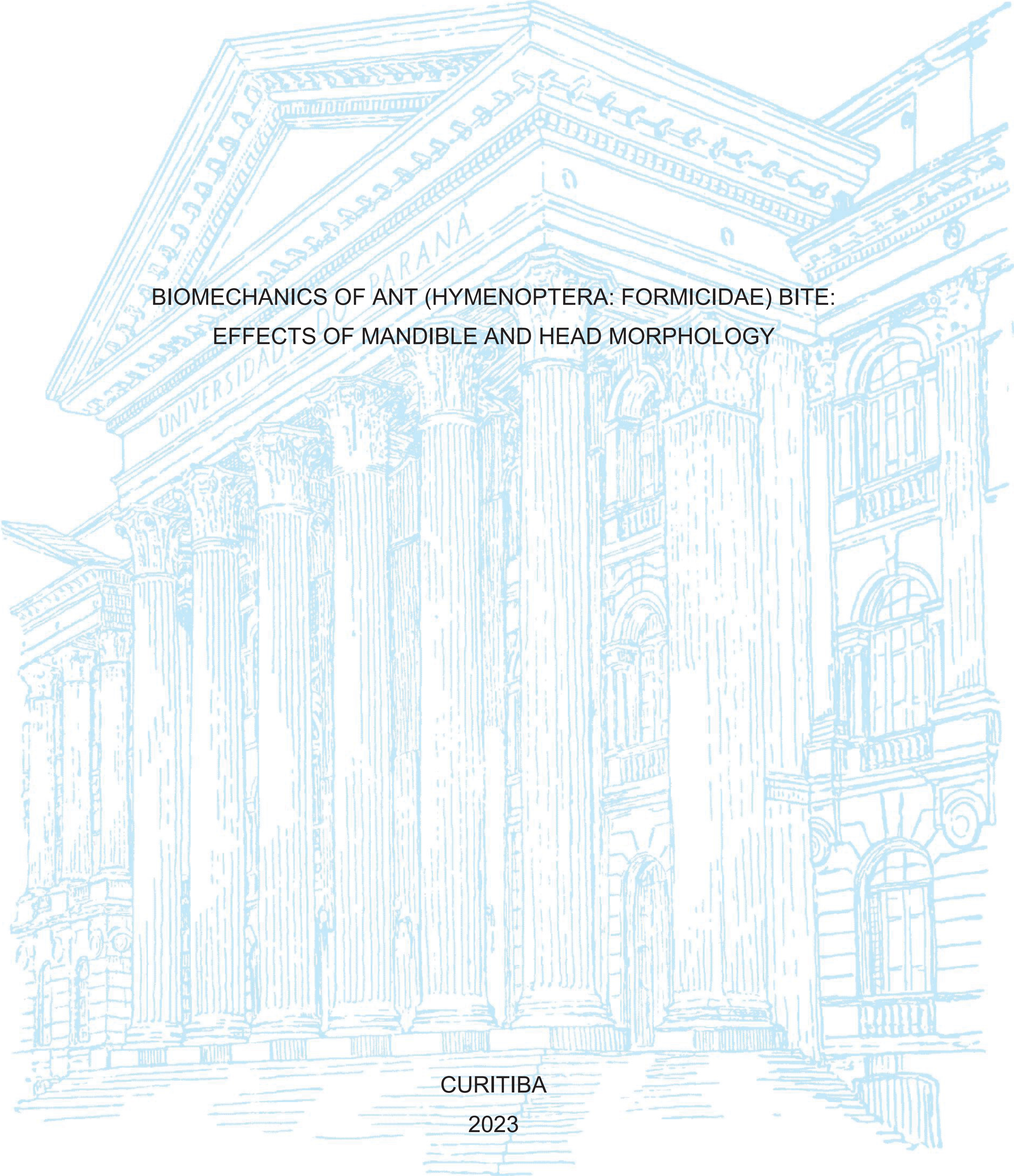

BIOMECHANICS OF ANT (HYMENOPTERA: FORMICIDAE) BITE: EFFECTS OF MANDIBLE AND HEAD MORPHOLOGY

CURITIBA
2023

CRISTIAN LUAN KLUNK

BIOMECHANICS OF ANT (HYMENOPTERA: FORMICIDAE) BITE: EFFECTS OF MANDIBLE AND HEAD MORPHOLOGY

Tese apresentada ao curso de Pós Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná (UFPR), e Departamento de Biologia, Technische Universität Darmstadt (TUD), como requisito parcial à obtenção do título de Doutor em Ecologia e Conservação (UFPR), e Doctor rerum naturalium (TUD).

Orientador: Prof. Dr. Marcio R. Pie (UFPR)

Co-orientador: Prof. Dr. Marco A. Argenta (UFPR)

Orientador: Prof. Dr. Michael Heethoff (TUD)

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **CRISTIAN LUAN KLUNK** intitulada: **Biomechanics of ant (Hymenoptera: Formicidae) bite: effects of mandible and head morphology**, sob orientação do Prof. Dr. MARCIO ROBERTO PIE, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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Dedico esta tese a todas e todos que dão suporte a educação pública no Brasil.

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Evolution might be labeled "the transformation of the possible". (GOULD S.J., 1981, Hyena Myths and Realities, p.11)

RESUMO

A necessidade de capturar e processar alimentos representa uma importante pressão evolutiva. Insetos são incrivelmente diversos, apresentando inúmeros hábitos alimentares e adaptações morfológicas relacionadas. Muitas linhagens de insetos evoluíram um hábito alimentar mastigador, onde as mandíbulas são apêndices essenciais para cortar, moer e rasgar itens alimentares que são adicionalmente processados pelas demais partes bucais. Mandíbulas também podem ser utilizadas para desempenhar outros comportamentos. Em formigas, as operárias utilizam as mandíbulas para executar a maior parte das atividades não reprodutivas da colônia, como carregar objetos e juvenis, escavar, cortar, moer e perfurar. Não surpreende, portanto, que essa dependência nas mandíbulas tenha gerado um significativo desenvolvimento dos músculos mandibulares das operárias de formigas, especialmente dos músculos adutores das mandíbulas, os maiores de uma operária de formiga. Esses músculos originam-se na cápsula da cabeça, cuja forma tem consequências relevantes para o armazenamento dos músculos. Formigas apresentam uma impressionante diversidade morfológica quanto à cabeça e mandíbulas, cujas implicações funcionais vem sendo investigadas sob distintas abordagens, porém usualmente restritas à espécies proximamente relacionadas. Algumas espécies de formigas apresentam diferenciações morfológicas entre as operárias, caracterizando um polimorfismo da casta operária com profundas implicações na divisão de trabalho da colônia. Simulações biomecânicas podem ser ferramentas efetivas para testar o desempenho funcional de uma estrutura sujeita à cargas externas. Em se tratando da cabeça e mandíbulas de operárias de formigas, a relevância funcional da variação morfológica observada nas diferentes linhagens, ou mesmo entre operárias da mesma espécie, pode ser investigada através de simulações que aproximem a mecânica da mordida. A Análise de Elementos Finitos (AEF) consiste num método numérico utilizado para aproximar as respostas mecânicas de uma estrutura submetida à cargas externas. Através de reconstruções digitais da estrutura de interesse é possível aplicar a AEF, considerando os aspectos mecânicos relevantes para o comportamento que se pretende simular, como uma mordida de formiga. O principal objetivo da presente tese é explorar a variação morfológica observada na cabeça e mandíbulas de formigas sob uma perspectiva funcional em dois níveis: intra e interespecificamente. Utilizamos a AEF para testar as respostas mecânicas dessas estruturas às demandas da mordida, comparando o desempenho funcional de operárias maiores e menores do gênero *Pheidole* no nível intraespecífico, e de diversas linhagens de formigas que compõem distintas subfamílias de formigas atualmente reconhecidas ao nível interespecífico. Nossos resultados sugerem que no gênero *Pheidole*, as operárias maiores possuem cabeças e mandíbulas melhor adaptadas para lidar com as demandas mecânicas da mordida, o que concorda com suas responsabilidades na colônia, já que são recrutadas para a defesa e processamento de alimento. Ao nível interespecífico, a variação morfológica também se mostrou relevante na resposta mecânica da cabeça e mandíbulas de formigas às demandas da mordida, embora apresentando relações incertas com características ecológicas das espécies. Nós demonstramos que a AEF é uma ferramenta valiosa para explorar aspectos funcionais da variação morfológica de formigas, e para gerar novas perspectivas sobre a evolução morfológica desses insetos.

Palavras-chave: Análise de elementos finitos. Divisão de trabalho. Mandíbulas. Morfologia funcional. Polimorfismo casta operária.

ABSTRACT

The necessity to capture and process food represents a relevant evolutionary pressure. Insects are incredibly diverse, showing several feeding modes and their associated morphological adaptations. Many insect lineages evolved a chewing feeding mode, where the mandibles are essential appendices to cut, crush, and tear food items that are further processed by other mouthparts. Mandibles can also be employed to perform additional behaviors. In ants, workers use their mandibles to execute most of the colony's non-reproductive tasks, like carry brood and objects, excavate, cut, crush, and punch. Not surprisingly, this heavy reliance on mandibles leads to a significant development of the mandibular muscles in ant workers, especially the mandibular closing muscles, the largest of an ant worker. Those muscles originate in the head capsule, whose shape has relevant consequences for muscle packing. Ants show an astounding head and mandible morphological diversity, whose functional implications have been investigated through distinct approaches, but usually regarding closely related species. Some ant species have further morphological variation among the workers, characterizing a worker polymorphism with profound implications for the colony division of labor. Biomechanical simulations can be effectively used to test a structure's functional performance under external loading. Concerning ant worker heads and mandibles, the functional relevance of the morphological variation observed in different lineages, or even among distinct worker types of the same species, could be investigated through simulations that resemble bite-loading conditions. Finite Element Analysis (FEA) is a numerical method used to approximate the mechanical responses of a structure to an external load. With digital reconstructions of the target structure, it is possible to perform an FEA, providing the relevant mechanical aspects of the behavior intended to simulate, like an ant bite. The main aim of this thesis is to explore the morphological variation observed in ant worker heads and mandibles under a functional perspective at two levels: intra and interspecifically. We employed FEA to test the mechanical responses of ant worker heads and mandibles under bite loading conditions, comparing the functional performance of major and minor workers of the dimorphic ant genus *Pheidole* at the intraspecific level, and of several ant lineages that span many of the currently recognized ant subfamilies at the interspecific level. Our results suggest that in the genus *Pheidole*, the major worker subcaste have heads and mandibles better suited to deal with the mechanical demands of bite, agreeing with its duties in the colony as workers responsible for defense and food processing. At the interspecific level, head and mandible morphology variation were also relevant in the mechanical responses of those structures to bite-loading demands, although with unclear relationships with ecological aspects. We demonstrated that FEA is a valuable tool to explore functional aspects of ant morphological variation and to provide insights into ant morphological evolution.

Keywords: Finite Element Analysis. Division of labor. Mandibles. Functional morphology. Worker polymorphism.

ZUSAMMENFASSUNG

Die Notwendigkeit, Nahrung zu verarbeiten, stellt einen relevanten evolutionären Druck dar. Insekten sind sehr vielfältig und weisen verschiedene Ernährungsweisen und die damit verbundenen morphologischen Anpassungen auf. Viele Insekten haben eine kauende Ernährungsweise entwickelt, bei der die Mandibeln wesentliche Anhängsel zum Schneiden, Zerkleinern und Zerreißen der Nahrung sind, die dann von anderen Mundwerkzeugen weiterverarbeitet werden. Die Mandibeln können auch für zusätzliche Verhaltensweisen eingesetzt werden. Bei Ameisen verwenden die Arbeiterinnen ihre Mandibeln, um die meisten nicht-reproduktiven Aufgaben der Kolonie auszuführen, wie das Tragen von Brut und Gegenständen, Ausgraben, Schneiden und Zerkleinern. Es überrascht nicht, dass diese starke Abhängigkeit von den Mandibeln bei den Ameisenarbeiterinnen zu einer verstärkten Entwicklung der Mandibelmuskulatur führt, die größten Muskeln einer Ameisenarbeiterin. Diese Muskeln haben ihren Ursprung in der Kopfkapsel, deren Form erhebliche Auswirkungen auf die Muskelmasse hat. Ameisen weisen eine erstaunliche morphologische Vielfalt von Kopf- und Mandibelformen auf, deren funktionelle Auswirkungen mit unterschiedlichen Ansätzen untersucht wurden. Bei einigen Ameisenarten gibt es weitere morphologische Unterscheidungen zwischen den Arbeiterinnen, die auf einen Arbeiterinnen-Polymorphismus mit tiefgreifenden Auswirkungen auf die Arbeitsteilung in der Kolonie zurückzuführen sind. Biomechanische Simulationen können effektiv eingesetzt werden, um die funktionelle Leistung einer Struktur unter externer Belastung zu testen. Bei den Köpfen und Mandibeln von Ameisenarbeiterinnen könnte die funktionelle Relevanz der morphologischen Variationen, die in verschiedenen Abstammungslinien oder sogar zwischen verschiedenen Arbeiterinnenarten derselben Spezies beobachtet wurden, durch Simulationen untersucht werden, die den Bedingungen einer Bissbelastung entsprechen. Die Finite-Elemente-Analyse (FEA) ist eine numerische Methode, mit der die mechanischen Reaktionen einer Struktur auf eine äußere Krafteinwirkung angenähert werden können. Mit digitalen Rekonstruktionen der Zielstruktur ist es möglich, eine FEA durchzuführen, die die relevanten mechanischen Aspekte des zu simulierenden Verhaltens, z. B. eines Ameisenbisses, liefert. Das Hauptziel dieser Arbeit besteht darin, die morphologische Variation der Köpfe und Mandibeln von Ameisenarbeiterinnen unter funktionalen Gesichtspunkten auf zwei Ebenen zu untersuchen: intra- und interspezifisch. Daher haben wir FEA eingesetzt, um die mechanischen Reaktionen von Ameisenarbeiterinnenköpfen und -mandibeln unter Bissbelastungsbedingungen zu testen und die funktionelle Leistung der dimorphen Ameisengattung *Pheidole* auf intraspezifischer Ebene und von mehreren Ameisenarten, die viele der derzeit anerkannten Ameisenunterfamilien umfassen, auf interspezifischer Ebene zu vergleichen. Unsere Ergebnisse deuten darauf hin, dass in der Gattung *Pheidole* die wichtigsten Arbeiterinnen-Unterkaste Köpfe und Mandibeln haben, die besser geeignet sind, um mit den mechanischen Anforderungen des Bisses umzugehen, was mit ihren Aufgaben in der Kolonie als Arbeiterinnen, die für die Verteidigung und die Nahrungsverarbeitung verantwortlich sind, übereinstimmt. Auf interspezifischer Ebene waren die Unterschiede in der Morphologie von Kopf und Mandibeln ebenfalls relevant für die mechanischen Reaktionen dieser Strukturen auf die Beißanforderungen, wenn auch mit unklaren Beziehungen zu ökologischen Aspekten. FEA ist demnach eine sehr gute geeignete Methode, um funktionelle Aspekte der morphologischen Variation von Ameisen zu untersuchen und Einblicke in die morphologische Evolution von Ameisen zu gewinnen.

Schlüsselwörter: Finite-Elemente-Analyse. Arbeitsteilung. Mandibeln. Funktionelle Morphologie. Arbeiterinnen-Polymorphismus.

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1.1 INTRODUÇÃO GERAL

A morfologia dos organismos evolui como resposta a diversas pressões. Relações entre forma e função são esperadas em muitos aspectos da história de vida dos organismos, e são investigadas sob várias abordagens metodológicas. Nos insetos, a evolução de distintos modos alimentares está relacionada com muitas adaptações morfológicas, envolvendo principalmente (mas não somente) as partes bucais (LABANDEIRA, 2019), que são apêndices da cabeça utilizados na captura, processamento e ingestão de alimentos (SNODGRASS, 1935). As partes bucais de insetos são ectognatas, o que significa que se articulam externamente à cabeça, sendo representadas pelo labro, hipofaringe, um par de mandíbulas e um de maxilas, bem como um lábio (CHAPMAN, 1998). Muitas linhagens de insetos possuem um aparato alimentar do tipo mastigador, onde as mandíbulas auxiliam na captura do alimento e em distintas formas de processar o alimento, como cortar, moer ou rasgar itens alimentares (BLANKE, 2019).

Boa parte das atuais linhagens de insetos apresentam mandíbulas dicondílicas, que articulam com a cabeça por meio de duas articulações do tipo bola-soquete, reduzindo o movimento mandibular a um único eixo de rotação, de modo que a abdução e adução são os únicos movimentos possíveis (BLANKE et al., 2015; BLANKE, 2019). Apesar dessa simplificação no movimento das mandíbulas, insetos com mandíbulas dicondílicas exibem uma vasta diversidade de formas de mandíbulas e hábitos alimentares. Embora a variação morfológica costuma ser associada com aspectos funcionais em insetos (WEIHMANN et al., 2015a, 2015b), as evidências de uma associação direta entre morfologia e hábito alimentar não são definitivas (BLANKE, 2017b; 2019). Em muitos insetos as mandíbulas desempenham diversas funções, o que compromete a especialização morfológica e pode ajudar a explicar a falta de associação entre morfologia da mandíbula e função. Isso é particularmente verdadeiro para formigas, especialmente a casta operária (WHEELER, 1910; WILSON, 1987).

Formigas são insetos eusociais que exibem uma divisão de trabalho reprodutiva. Membros alados da colônia, as rainhas e machos, são responsáveis pela reprodução, enquanto que os indivíduos não alados, as operárias, desempenham as atividades não reprodutivas essenciais para a manutenção da colônia (WILSON, 1971; HÖLLDOBLER; WILSON, 1990). Para desempenhar suas tarefas diárias as operárias utilizam basicamente suas mandíbulas, o que as permite morder, transportar objetos, escavar, processar alimento, e outras funções (WHEELER, 1910; WILSON, 1987). Essa dependência das operárias nas suas mandíbulas está associada a um desenvolvimento significativo dos músculos adutores das mandíbulas, o *M. craniomandibularis internus* (0md1), que ocupa boa parte do volume interno da cápsula da cabeça. Um reduzido par de músculos, o *M. craniomandibularis externus* (0md3), é responsável pela abdução das mandíbulas (PAUL; GRONENBERG, 2002; LILLICO-OUACHOUR et al., 2018; RICHTER et al., 2019; 2020; 2021; BOUDINOT et al., 2021; KATZKE et al., 2022). Devido às limitações no movimento das mandíbulas, operárias de formigas precisam desempenhar suas funções modulando a força e velocidade de suas mordidas no seu único plano de ação, além de utilizar as demais partes bucais para auxiliar no processamento de alimentos. Essa modulação é alcançada pela versatilidade do 0md1, composto por vários conjuntos de fibras musculares que variam em suas características contráteis (GRONENBERG et al., 1997; PAUL, 2001). Algumas fibras musculares possuem sarcômeros longos que geram lentas mas potentes contrações, enquanto outras consistem de sarcômeros curtos que geram contrações rápidas, porém tênues, sendo a proporção dessas fibras no 0md1 variável entre espécies (PAUL; GRONENBERG, 1999). Ainda, em algumas linhagens de formigas evoluíram mecanismos especializados de amplificação de potência, onde pequenas alterações morfológicas, especialmente nas articulações das mandíbulas com a cabeça, formam um sistema mecânico onde a contração do 0md1 armazena energia potencial que é subitamente liberada na forma de velozes estalos das mandíbulas. Esse fenômeno resulta em movimentos mais rápidos do que o possível pela simples contração muscular (GRONENBERG, 1995; 1996; GRONENBERG et al., 1998; LARABEE et al., 2017; 2018; GIBSON et al., 2018; BOOHER et al., 2021).

Dada a relevância do 0md1 nas operárias de formigas, é possível supor que sua contração exerça demandas mecânicas significativas na cápsula da cabeça das operárias, sendo que a distribuição das tensão geradas pela contração do 0md1 potencialmente varia com a forma da cabeça. A força da mordida em insetos está diretamente relacionada com o volume dos músculos adutores das mandíbulas (BLANKE, 2019; RÜHR et al., 2022), e músculos maiores estão associados a demandas mecânicas mais elevadas na cápsula da cabeça. Além disso, mordidas mais potentes implicam em forças de reação das mandíbulas mais elevadas, o que também impacta a cápsula da cabeça através das articulações das mandíbulas. Diversos estudos sugerem que a forma da cabeça tem implicações relevantes para a alocação do 0md1 em operárias de formigas

(PÜFFEL et al., 2021; 2023a; 2023b, PAUL; GRONENBERG, 1999; PAUL, 2001). Todavia, como a forma da cabeça está associada com a capacidade de lidar com as demandas mecânicas da mordida ainda é pouco conhecido. Evidências obtidas em estudos de linhagens mais basais de insetos sugerem que a morfologia externa e interna da cabeça possui efeitos relevantes para a capacidade da cápsula da cabeça de lidar com as demandas da mordida (BLANKE et al., 2017a; 2018).

Além da variação interespecífica na morfologia da cabeça e mandíbulas, muitas espécies de formigas chamam atenção pela variação morfológica entre indivíduos da mesma colônia. Em algumas linhagens de formigas, além das diferenças morfológicas entre os indivíduos reprodutivos e não reprodutivos, a casta operária exibe algum nível de diferenciação morfológica, culminando em distintos tipos de operárias. Em formigas monomórficas, apenas pequenas variações de tamanho são observadas entre as operárias. Todavia, há casos em que o crescimento alométrico resulta em dois ou mais tipos de operárias discretamente reconhecidas, ou mesmo variações contínuas ao longo de um gradiente, representando níveis distintos de polimorfismo (WILSON, 1953). Diversos estudos exploraram os determinantes genéticos (GADAGKAR, 1999; HUANG et al., 2013), ecológicos (POWELL; FRANKS, 2005; 2006; POWELL, 2009) e de desenvolvimento (WHEELER, 1991; RAJAKUMAR et al., 2018) do polimorfismo da casta operária em distintas linhagens de formigas (FJERDINGSTAD; CROZIER, 2006; WILLS et al., 2018), sendo sugerido que o polimorfismo da casta operária pode aprimorar a divisão de trabalho e a eficiência na execução de tarefas na colônia, pois permite que operárias morfologicamente distintas se especializem em diferentes tarefas (OSTER; WILSON, 1978; WILLS et al., 2018).

Uma maneira efetiva de investigar o papel da morfologia das mandíbulas e cabeça no contexto das demandas mecânicas da mordida é desenvolvendo simulações biomecânicas em representações digitais dessas estruturas. A Análise de Elementos Finitos (AEF) é uma abordagem analítica amplamente utilizada para esse fim. Essa análise consiste num método numérico que aproxima a resposta mecânica de estruturas (por exemplo, tensão e deformação) à cargas externas (KIM et al., 2018). De maneira geral, para implementar a AEF é necessário um modelo digital da estrutura de interesse, que pode ser representado por elementos uni, bi ou tridimensionais que são agrupados numa malha de elementos finitos (RAYFIELD, 2007; KUPCZIK, 2008). Para simular apropriadamente o comportamento de interesse, é preciso especificar regiões da malha onde as cargas serão aplicadas, bem como partes onde deslocamentos não serão permitidos, que representam pontos de contato da estrutura com o restante do corpo (RAYFIELD, 2007; KUPCZIK, 2008). Ainda, as propriedades mecânicas do material que compõe a estrutura precisam ser informadas, visto que elas descrevem o comportamento mecânico do material. Valores mais precisos das propriedades mecânicas geram resultados numéricos mais acurados da resposta da estrutura. Usualmente as propriedades mecânicas exigidas para aplicar a AEF são o módulo de Young e o coeficiente de Poisson. O módulo de Young mede a resistência do material à deformação elástica (LABONTE et al., 2017). O coeficiente de Poisson, por sua vez, mede a razão de deformação lateral/longitudinal sob um carregamento de tração (HIBBELER, 2017). Embora relevantes para descrever as características mecânicas do material, a quantificação dessas variáveis é complexa, havendo poucos dados de tecidos biológicos disponíveis (VINCENT; WEGST, 2004), com raras medições realizadas da cutícula de formigas (SCHOFIELD et al., 2021; BRITO et al., 2017; Li et al., 2020). Entretanto, quando somente os efeitos da variação na forma da estrutura são de interesse, como numa abordagem comparativa, é possível fornecer valores genéricos e únicos das propriedades materiais das estruturas de diferentes espécies (RAYFIELD, 2007).

Para testar os efeitos da variação morfológica intra e interespecífica da cabeça e mandíbulas de operárias de formigas sob as demandas da mordida, aplicamos a AEF em representações 2D e 3D dessas estruturas em diferentes condições biomecânicas. Exploramos a diversidade morfológica de formigas numa perspectiva funcional, relacionando a morfologia e desempenho mecânico das mandíbulas e cabeça com características comportamentais e ecológicas das espécies e tipos de operárias. Ao longo dos cinco capítulos dessa tese, exploramos perguntas relativas a dois níveis da diversidade morfológica de formigas. Ao nível intraespecífico focamos no gênero *Pheidole*, que possui uma casta operária dimórfica e, em alguns casos, trimórfica (WILSON, 2003; MOREAU, 2008). Ao nível interespecífico, exploramos a variação morfológica de diversas espécies de formigas, examinando a diversidade morfológica desse grupo de insetos. Detalhes dos procedimentos adotados para cada conjunto de perguntas são providos em cada capítulo, bem como os objetivos, hipóteses e informações relacionadas que servem de base para cada capítulo. Informações gerais que baseiam a tese, conforme exposto nessa introdução no que se refere aos aspectos básicos da mecânica da mordida em formigas, bem como a importância da mordida para as atividades das operárias, não serão detalhados para cada capítulo.

1.2 GENERAL INTRODUCTION

Organismal morphology evolves as a response to several pressures. Form-function relationships are expected in several organism life history aspects and are investigated under many methodological approaches. In insects, the evolution of distinct feeding modes relates to many morphological adaptations, involving mainly (but not only) the mouthparts (LABANDEIRA, 2019), which are head appendices employed in the capture, processing, and ingestion of food (SNODGRASS, 1935). Insect mouthparts are ectognathous, meaning they articulate externally with the head, being represented by the labrum, a hypopharynx, a pair of mandibles and maxillae, and a labium (CHAPMAN, 1998). Many insect lineages possess a chewing feeding apparatus, where the mandibles usually aid in food capture and in distinct ways of food processing, like cutting, milling, or tearing apart the food sources (BLANKE, 2019).

Many current insect lineages have dicondylic mandibles that articulate with the head through two ball-and-socket joints, reducing mandibular movement to a single axis of rotation, so that abduction and adduction are the only possible movements (BLANKE et al., 2015; BLANKE, 2019). Despite this simplification in mandibular movement, insects with dicondylic mandibles exhibit a vast diversity of feeding habits and mandible morphologies. While insect mandibular morphological variation is often associated with functional aspects (WEIHMANN et al., 2015a, 2015b), there is no compelling evidence that such an association is a frequent evolutionary pattern (BLANKE, 2017b; 2019). In several insects the mandibles perform many functions, which compromises specialization and may underline the lack of association between mandible morphology and functional aspects. This is particularly true for ants, especially the worker caste (WHEELER, 1910; WILSON, 1987).

Ants are eusocial insects that exhibit a reproductive division of labor. Winged members of the colony, namely queens and males, are responsible for reproduction, while the wingless individuals, or workers, perform the non-reproductive tasks essential for colony maintenance (WILSON, 1971; HÖLLDOBLER; WILSON, 1990). To carry out their daily tasks workers rely heavily on their mandibles, which enable them to bite, transport objects, dig, process food, and perform other functions (WHEELER, 1910; WILSON, 1987). The significant reliance on mandibles for task performance has led to the crucial development of the mandibular adductor muscle *M. craniomandibularis internus* (0md1) in workers, occupying most of the head’s internal volume. A much smaller pair of muscles, the *M. craniomandibularis externus* (0md3), is responsible for mandible abduction (PAUL; GRONENBERG, 2002; LILLICO-OUACHOUR et al., 2018; RICHTER et al., 2019; 2020; 2021; BOUDINOT et al., 2021; KATZKE et al., 2022). Due to mandible movement limitations, ant workers must perform various tasks by modulating the force and velocity of their bites, along with employing other mouthparts to further process food. This modulation is accomplished through the versatility of the 0md1 muscle, composed of several sets of fibers that vary in their contractile characteristics (GRONENBERG et al., 1997; PAUL, 2001). Some muscle fibers have long sarcomeres that generate slow but powerful contractions, whereas others consist of short sarcomeres that produce fast but weaker contractions, and the proportion of these distinct fiber types in the 0md1 also varies interspecifically (PAUL; GRONENBERG, 1999). In some specialized ant species, power amplification mechanisms have evolved in which subtle morphological modifications, mainly in the mandible articulations with the head, generate a mechanical system where the contraction of the 0md1 stores potential energy that is suddenly released to generate high-speed strikes. This results in faster movements than possible under muscle contraction only (GRONENBERG, 1995; 1996; GRONENBERG et al., 1998; LARABEE et al., 2017; 2018; GIBSON et al., 2018; BOOHER et al., 2021).

Given the prominence of 0md1 in ant workers, we can expect that muscle contraction exerts significant mechanical demands on the worker's head capsule, with stress patterns generated by the 0md1 contraction potentially varying according to head shape. Bite force capacity in insects is directly related to the volume of the mandibular closing muscles (BLANKE, 2019; RÜHR et al., 2022), and larger muscles are associated with higher loading demands on the head capsule. Moreover, forceful bites imply higher mandibular reaction forces, which also impact the head capsule through the mandibular articulations with the head. Several studies suggested that head shape can have relevant implications for the mandibular closing muscles allocation in ant workers (PÜFFEL et al., 2021; 2023a; 2023, PAUL; GRONENBERG, 1999; PAUL, 2001). However, how head morphology associates with the capacity to deal with the mechanical demands of bite is still poorly investigated. Evidence gathered from basal insect lineages suggests that head external and internal morphology have relevant effects on the ability of the head capsule to deal with bite loading (BLANKE et al., 2017a; 2018).

Besides the interspecific variation of the mandible and head morphology observed in ants, several species draw attention due to further morphological distinctions among individuals of the same colony. Beyond the differences between the reproductive and worker individuals, the worker caste further differentiates into morphologically distinct worker types in some ant lineages. In monomorphic ants, only subtle variation in body size is observed among the workers. However, there are cases where allometric scaling results in two or more recognizable discrete worker types (species often called dimorphic, trimorphic, and so on) or even continuous variation along a gradient, representing different levels of worker polymorphism (WILSON, 1953). Several studies explored the genetic (GADAGKAR, 1999; HUANG et al., 2013), ecological (POWELL; FRANKS, 2005; 2006; POWELL, 2009), and developmental (WHEELER, 1991; RAJAKUMAR et al., 2018) determinants of worker polymorphism in distinct ant lineages (FJERDINGSTAD; CROZIER, 2006; WILLS et al., 2018), and it is suggested that worker polymorphism can improve colony division of labor and task efficiency by allowing different worker types to specialize in distinct colony tasks (OSTER; WILSON, 1978; WILLS et al., 2018).

An effective way to investigate the role of the mandible and head morphology in the context of bite-loading demands is to perform biomechanical simulations in digital representations of such structures. An analytical approach widely used for this end is Finite Element Analysis (FEA). This analysis is a numerical method that approximates the mechanical responses of structures (*e.g.* stress, strain, displacement) to external loading (KIM et al., 2018). In a general sense, to be able to implement the FEA, a digital representation of the structure of interest is needed, which can be represented by 1D, 2D or 3D elements that are assembled in a finite element mesh (RAYFIELD, 2007; KUPCZIK, 2008). To properly simulate the behavior of interest, it is necessary to specify regions of the mesh where loads are applied, as well as parts where no displacements are allowed, representing points of contact of the structure with the remaining of the body (RAYFIELD, 2007; KUPCZIK, 2008). In addition, the mechanical properties of the structure material need to be provided, which describes its mechanical behavior. More reliable mechanical properties result in more accurate numerical estimates of the structure's mechanical responses. Usually, the mechanical properties required to perform an FEA are the Young's modulus and the Poisson ratio. Young's modulus measures the material resistance to elastic deformation (LABONTE et al., 2017). The Poisson ratio, otherwise, measures the material ratio of lateral to longitudinal deformation under tensile loading (HIBBELER, 2017). Although relevant to describe the mechanical characteristics of a material, such variables are difficult to measure, with little data available for biological tissues (VINCENT; WEGST, 2004), and measurements on ant cuticles were rarely performed (SCHOFIELD et al., 2021; BRITO et al., 2017; Li et al., 2020). However, when the interest is solely on the effects of variation in the structure morphology, like in a comparative approach, it is possible to provide generic and single values of the material properties for the different structures (RAYFIELD, 2007).

To test for the effects of intra and interspecific morphological variation of ant worker mandible and head under bite loading, we applied the finite element method in 2D/3D digital models of such ant structures under different biomechanical conditions. We explored the morphological diversity of ant workers considering a functional perspective, relating the morphology and mechanical performance of ant heads and mandibles to behavioral and ecological peculiarities of the different worker types and ant species. Along the five manuscripts of this thesis, we explored questions regarding two main levels of ant worker morphological diversity. At the intraspecific level we focused on the genus *Pheidole*, which shows a dimorphic and, in a few cases, trimorphic worker caste (WILSON, 2003; MOREAU, 2008). At the interspecific level, we considered many ant species to explore the morphological diversity observed in this insect group. More specific details of the procedures adopted to answer each set of questions are provided in each manuscript, as well as the aims, hypothesis, and related information that substantiate them. General aspects that substantiate this thesis, like the description of the basics of ant bite mechanics as stated in this introduction, along with the importance of the mandibles to ant worker colony tasks, will not be repeated in each manuscript.

2. MANUSCRIPT 1

Mandibular morphology and task specialization in *Pheidole* (Hymenoptera: Formicidae)

Results from this manuscript were published in the Journal *Journal of the Royal Society Interface* (KLUNK, C.L., ARGENTA, M.A.; CASADEI-FERREIRA, A.; ECONOMO, E.; PIE, M.R. Mandibular morphology, task specialization and bite mechanics in *Pheidole* ants (Hymenoptera: Formicidae). **Journal of The Royal Society Interface**, v. 18, n. 179, p. 20210318, jun. 2021)

A.C.F. and E.P.E. collected the raw data; C.L.K., A.C.F., M.A.A. and M.R.P. contributed on the study design; C.L.K., A.C.F. and M.A.A. contributed on data analysis; C.L.K. and M.R.P. drafted the manuscript; A.C.F., E.P.E. and M.A.A. critically revised the manuscript; M.R.P. coordinated the study.

2.1 Abstract

Ants show remarkable ecological and evolutionary success due to their social life history and division of labour among colony members. In some lineages, the worker force became subdivided into morphologically distinct individuals (i.e. minor versus major workers), allowing for the differential performance of particular roles in the colony. However, the functional and ecological significance of these morphological differences are not well understood. Here, we applied finite element analysis (FEA) to explore the biomechanical differences between major and minor ant worker mandibles. Analyses were carried out on mandibles of two *Pheidole* species, a dimorphic ant genus. We tested whether major mandibles evolved to minimize stress when compared to minors using combinations of the apical tooth and masticatory margin bites under strike and pressure conditions. Majors performed better in pressure conditions yet, contrary to our expectations, minors performed better in strike bite scenarios. Moreover, we demonstrated that even small morphological differences in ant mandibles might lead to substantial differences in biomechanical responses to bite loading. These results also underscore the potential of FEA to uncover biomechanical consequences of morphological differences within and between ant workers.

2.2 INTRODUCTION

The evolution of complex societies in ants followed the advent of reproductive division of labour into distinct castes, in which largely sterile and wingless individuals (i.e. workers) perform quotidian colony tasks, whereas winged individuals became specialized for colony reproduction (i.e. queens and males) (WILSON, 1971; HÖLLDOBLER; WILSON, 1990). These changes were accompanied by substantial morphological differences among reproductives and non-reproductives, with the latter giving up reproduction and dispersal capacities while experiencing both morphological and behavioral specialization (KELLER et al., 2014; DIVIESO et al., 2020; PEETERS et al., 2020). In some ant lineages, the worker force became further subdivided into morphologically distinct subcastes (e.g. minor versus major workers), and such differences are thought to allow differential performance of particular roles in the colony, such as seed milling and defense (WILSON, 1953; OSTER; WILSON, 1978).

The genus *Pheidole* have a dimorphic worker caste, represented by major and minor workers, where majors have a disproportionately larger head (WILSON 1953; RAJAKUMAR et al., 2018; WILSON, 2003). Along with those morphological aspects, workers of *Pheidole* also show distinct roles in the colony. Minors led most of the colony non-reproductive tasks (e.g. foraging, brood care, nest maintenance), whereas majors are mostly recruited to food processing and defense (WILSON, 1984; MERTL; TRANIELLO, 2009). *Pheidole* species are distributed worldwide, but most of their diversity and abundance is concentrated in the tropics (ECONOMO et al., 2015b; 2019). Although *Pheidole* species are typically considered diet generalists (WILSON, 2003), some species might show some degree of dietary specialization (ROSUMEK, 2017). Of all their food items, feeding on seeds evolved many independent times and has been indicated as an important factor to explain the lineage diversification due to behavioral and morphological adaptations related to seed harvesting and processing (MOREAU, 2008). Since majors are specialized in tasks such as defense and food processing (WILSON, 1984; 2003), their larger heads could be a consequence of evolutionary pressures towards the specialization to those tasks (PIE; TRANIELLO, 2007). However, evidence gathered so far has been mixed (*e.g.* HOLLEY et al., 2016).

Understanding the main trends in the morphological evolution of *Pheidole* has received considerable attention in the past decades, with contrasting results to the relative contributions of size and shape to the morphological diversity of the genus (PIE;

TRANIELLO, 2007; PIE; TSCHÁ, 2013; SARNAT et al., 2017; FRIEDMAN et al., 2019; 2020). Regardless of the importance of mandibles to many aspects of ant life history, little is known about how morphological variation between species or worker subcastes relates to bite loading demands, except for one specialized snap-jaw species (LARABEE et al., 2018). Worker polymorphism can lead to behavioral specialization, mainly through variation in mandible morphology (HUANG, 2012; CAMARGO et al., 2015; SILVA et al., 2016), but biomechanical approaches to directly assess this relationship in ants are scarce (LARABEE et al., 2018). To understand how mandible morphology relates to the biomechanical demands of biting, it is important to employ approaches that allow for the direct assessment of bite loading conditions, as the Finite Element Analysis (FEA). By employing FEA, one an assess how variation in mandibular morphology among ant species as well as between castes and subcastes translates into the capacity of mandibles to deal with bite loading demands (LARABEE et al., 2018), as also explored for the evolution of mandible form in dragonflies (BLANKE et al., 2017b), stag beetles (GOYENS et al., 2014; 2015; 2016), and the functional morphology of the mouthparts of the reticulated beetle *Priacma serrata* (LeConte) (HÖRNSCHEMEYER et al., 2013). Biomechanical approaches employing FEA have also revealed important aspects of the evolution of other insect structures, such as wings and the mechanics of flight (RAJABI et al., 2015; 2016) and the evolution of insect head capsules (BLANKE et al., 2017a; 2018).

To improve our understanding of morphological evolution in *Pheidole* species, and the role of morphological differentiation to improve task specialization in polymorphic ants, we simulate several bite scenarios *in silico* by applying FEA (RAYFIELD, 2007; KUPCZIK, 2008) on three-dimensional (3D) models of minor and major mandibles of two *Pheidole* species. We hypothesize that major mandibles are better able to mitigate stress than those of minors, given their greater robustness. Alternatively, if each worker subcaste has mandibles optimized to perform different tasks, majors and minors could perform better in distinct biting scenarios. Interspecific differences are expected between the more distinct mandibles of majors, which can suggest changes in the capacity to deal with hard food items, given the specialized roles of those workers (WILSON, 1984; 2003). Alternatively, differences between species in minor worker mandibles will suggest that even small morphological distinctions can lead to biomechanical idiosyncrasies.

2.3 MATERIALS AND METHODS

2.3.1 *Studied species*

We considered the major and minor workers of *Pheidole hetschkoi* Emery and *P*. cf. *lucretii* to test the influence of mandible shape in biting performance. Majors of *P*. *hetschkoi* are sturdy, with larger heads and more robust mandibles than *P*. cf. *lucretii* majors, which have also smaller heads and are slender. Minors differ little between species in mandible shape. *Pheidole hetschkoi* harvest and accumulate seeds in their nests, which was never recorded for *P*. cf. *lucretii* (personal observation).

2.3.2 *CT scanning and image processing*

One specimen of each subcaste and species were scanned with a ZEISS Xradia 510 Versa X-ray microCT scanner at the Okinawa Institute of Science and Technology, using the software ZEISS Scout and Scan Control System. Exposure time of each specimen varied from 1 to 5 s, under an ‘Air’ filter and 4× objective. The voltage was set between 30 and 50 keV, from 4 to 5 W of power, under a ‘normal’ field mode and intensity levels of 15 000 and 17 000 across the whole specimen. Scan time varied from 27 to 30 min, generating 801 projections from full 360-degree rotations. Model reconstruction was performed with XMReconstructor, and mandibles segmentation was carried in ITK-snap 3.8.0 (YUSHKEVICH, 2006). For mesh generation and simplification, we used the software MeshLab (CIGNONI, 2008), and to generate 3D mandible models for FEA simulations, we used the software Fusion 360 (AUTODESK). Ant mandibles are internally hollow, and their cuticle varies in thickness along the mandible axis, characteristics that can influence mechanical behaviour and structure stiffness, and we incorporated these aspects in our 3D representations to model realistic mandible morphologies.

2.3.3 *Finite element analysis simulations*

FEA is a numerical method that approximates the mechanical responses of a structure submitted to loading demands (KUPCZIK, 2008) that, in the case of biological structures, could represent the demands of biting, running, jumping, and so on (RAYFIELD, 2007). Here, the structures of interest are the *Pheidole* workers mandibles, and the loadings demands refer to the loadings of different bite conditions. To quantify the mechanical response of a structure to external loading, FEA requires the discretization of the structure into small parts, resulting in the finite element mesh composed of elements of pre-defined shape and a specific number of points, called nodes, used to solve the equations (BATHE, 1995, MARCÉ-NOGUÉ et al., 2015). Displacements on nodes are calculated to estimate stress and strain, based on the structure material properties and shape (RAYFIELD, 2007). We used 10-node tetrahedral elements (C3D10) to generate the finite element mesh. The number of elements varied for each model, as well as the size of each element between subcastes, to adapt meshes to each morphology (Table 1).

We performed linear static simulations of four distinct biting scenarios for each species and subcastes, divided into two categories, namely strike and pressure, which reflect different aspects of mandible movement in terms of force and velocity. In all simulations, we defined the constrained and loaded regions in a way to capture the mechanical response at the exact moment that the mandible hits or presses an object. Therefore, we did not intend to simulate the conditions during the mandibular closing movement. In strike scenarios, a condition associated with faster movements, we define the mandible articulations with the head (dorsal - dma and ventral - vma) as the constrained regions, applying static load on the apical tooth or the masticatory margin (at and mm, Figure 1a). In pressure scenarios, associated with slower mandible movements but powerful bites, in addition to the mandibular joints, we also constrained the apical tooth or the mm and applied the load to the region of 0md1 insertion, following the direction of contraction (Figure 1b) to simulate the use of mandibles for food compression. We constrained nodal displacement in x, y, and z directions and apply a 1 N load uniformly distributed among nodes in all simulations. We modeled the mandible cuticle as an isotropic and linearly elastic material, setting Young’s modulus as 2.75 GPa and the Poisson’s ratio as 0.3, based on measures from the cuticle of ant mandibles available in the literature (BRITO et al., 2017). Given that we intended to investigate how variation in mandible morphology affects the mechanical responses to the same loading demands in different biting conditions, the only source of variation for each biting simulation between species and workers was the morphology of the mandibles. Therefore, we can test if some morphologies are better suited for specific biting conditions. We present FEA stress results based on Tresca failure criterion, more suitable for brittle fracture, which determines an equivalent stress value under which the material will possibly fail when subjected to combined load (ÖZKAYA et al., 2017). We used Abaqus 6 (Dassault Systèmes) to run the FEA simulations.

2.4 RESULTS

2.4.1 *FEA simulations*

Stress distribution results are shown in Figure X. Given that the volume of each model varies, and that we use idealized loads and material properties, we chose not to interpret absolute stress values. Rather, we will focus on qualitative differences among simulations by rescaling the stress ranges based on a reference model to facilitate comparisons between species, subcastes, and biting scenarios. Therefore, relative differences in stress distribution between simulations indicate mandibular biomechanical distinctions to assimilate loading conditions.

2.4.2 *Major worker mandibles*

When displacement restrictions were applied on the mandibular joints, those regions expectedly showed high-stress levels, but stresses had to spread to other regions to be effectively absorbed. Starting from the dma, stresses dissipate mainly along the mandible external face (ef) and trulleum (tr, Fig. 2c, d, g, h, k, l, o, p). Indeed, the trulleum and the canthellus (ca, Fig. 2a, b, c, d) were important to concentrate stresses coming from the dma in all simulations. Stresses from the vma spread mainly along the external margin (em) and through its surroundings along the internal (if) and external faces of the mandible (Fig. 2c, d, g, h, k, l, o, p). Contrasting different biting scenarios, higher stresses are found when only the apical tooth is employed (Fig. 2c, d, k, l). This result indicates that ants face marked mechanical restrictions whether they only use the apical tooth. Pressure scenarios generated higher stresses around the basal region of the if (Fig. 2k, l, o, p), whereas strike scenarios concentrated more stress near the mm, an expected consequence of load application (Fig. 2c, d, g, h). However, the key aspect relating to different biting scenarios are the higher stress levels in dma and vma in the strike (Fig. 2c, d, g, h) versus pressure simulations (Fig. 2k, l, o, p), which indicates that strike causes higher mechanical demands in the mandibular joints than pressure.

The main aspect that influences stress dissipation differences between species is the presence of a groove in the if. When applying a load or constraining the mm of majors, the mm concavity (defined by the masticatory margin and a parallel carina ventrolaterally) of both species concentrate stress, but much of the stress spreads in direction to the if.

Pheidole hetschkoi has a deeper groove near the mm, which acts as an important stress concentrator, mainly in strike scenarios on the mm (ifg, Fig. 2g). While *P*. cf. *lucretii* also shows stress concentration at the same region in this biting scenario, those stresses spread more extensively along the if (Fig. 2h), which suggests that its groove is shallow and does not act as a stress concentrator. The ef curvature also differs between species, but there are no substantial differences in stress dissipation pattern (Fig. 2c, d, g, h, k, l, o, p). The dissipation through the ef is more restricted to the articulations surroundings, given the robustness of the mandibular base, which could explain why there is not a conspicuous effect of the ef curvature in the stress dissipation pattern between species. Stresses were proportionately higher in the *P*. cf. *lucretii* mandible, through most mandibular regions and all biting scenarios, but the differences are more striking in pressure scenarios (Fig. 2l, p).

2.4.3 *Minor worker mandibles*

There is a distinguished stress concentration around the more constricted region of the if, a trend that occurs mainly in strike simulations, especially when the load was applied on the mm (Fig. 2e, f). This constriction acts as a stress concentrator in minors due to their slender mandibles in comparison to majors. When the results of different species are compared, P. cf. lucretii simulations show proportionately higher stresses than *P*. *hetschkoi* in general (Fig. 2b, f, j, n), contrary to the expectation that minors mandibles would not differ in mechanical performance. The overall lower stress levels found in mm strike simulations of *P*. *hetschkoi* minor seems to reflect the presence of well-developed teeth along its mm. It is noticeable that the mm teeth absorb great levels of stress (Fig. 2e) so that their absence leads to higher stress levels along the mandible surfaces in strike simulations of *P*. cf. *lucretii* minor, as well as in majors of both species. The higher stresses along the if in *P*. cf. *lucretii* minor mandible, compared to *P*. *hetschkoi* minor mandible, draw attention to the mechanical limitations associated with worn mandibles, as is the case of the *P*. cf. *lucretii* minor mandible modeled, which can lead to behavioral switches in task performance along the worker lifetime. Regarding the biting scenarios, pressure in minors results in higher stresses on the mandibular internal and external faces of both species when compared to majors (Fig. 2i, j, m, n). As occurred in pressure scenarios for majors, stresses along the if concentrate near the mandible base, where the load was applied. However, in minors, the mandible base is slender, which can explain why the mandibular surfaces in minors are proportionately more stressed in pressure than in strike simulations.

2.5 DISCUSSION

In this study, we apply FEA in mandibles of *Pheidole* workers to simulate different biting scenarios and investigate how morphological differences in mandible morphology reflect their responses to those bite loading demands. Our results demonstrate how the mandible morphology of dimorphic workers can be optimized for particular tasks and draws attention to the role of specific mandibular regions or structures to deal with the stresses generated by their bite. Most ant species have a triangular shaped mandible (SNODGRASS, 1935; RICHTER et al., 2019), which can be divided into two components, a basal thick stem, and a distal blade (RICHTER et al., 2020). Our results indicate that the increased thickness of the mandible basal region may conform to the high loading demands experienced by the mandibular articulations with the head. Most of the stresses generated on the apical tooth dissipate along the external margin towards the mandibular base, in both species and subcastes, avoiding the spread of considerable stresses through the more delicate mandibular surfaces. In strike simulations on the masticatory margin, the presence of well-developed teeth results in stresses being concentrated on the teeth instead of spreading through the internal face of the mandible. Majors of *Pheidole*, in which the masticatory margin is toothless, show high levels of stress in the masticatory margin concavity that are not entirely absorbed in this region. Interestingly, they have a deeper groove on their mandible internal face, especially *P. hetschkoi*, which helps to concentrate stresses near the more robust masticatory margin instead of spreading through the internal face of the mandible. Although alleviating the level of stress in the mandibular articulations, such stress concentration can be harmful in cases in which the structure is submitted to cycles of loading, leading to structural failure due to material fatigue (DIRKS et al., 2013).

An important aspect of *Pheidole* mandibular morphology to bite mechanics is the role of the trulleum and the canthellus on stress concentration. The trulleum is a concavity near the *dma* that is delimited by a cuticular ridge called canthellus, a configuration that is present only in some myrmicine ants (RICHTER et al., 2019). The function of the trulleum was hitherto unknown, although it was suggested that it could act as an additional stabilization of the mandible (RICHTER et al., 2020). Here we demonstrate for the first time the importance of the trulleum and the canthellus to assist in stress concentration along the *dma*, avoiding the spread of stresses through the more delicate mandibular surfaces. This is an interesting discovery, given that the *dma* seems to concentrate higher stresses in general than the *vma*. Given the suggested functional role of those mandibular regions outlined by our results, it would be interesting to investigate the biomechanical responses of mandibles that lack the development of this configuration to understand how stresses dissipate from *dma* without those important stress concentrators, especially in ant species with similar loading demands as *Pheidole* mandibles. Although many ant lineages share the common mandible triangular shape as *Pheidole* species (RICHTER et al., 2019), other subtle morphological characteristics could assist in stress concentration (e.g., the cuticular thickness around this region and the mandible curvature pattern). Differences in mandible use and diet can also influence the amount of stress in the mandibular articulations (e.g., a diet rich in liquid food represents much lower bite loading demands than one composed of seeds or arthropods).

Our results also underscore how the more robust major mandibles are better suited to deal with pressure biting than minors' slender mandibles, which surprisingly show higher performance in strike scenarios. These results agree with the specialized roles played by major workers in the colony. The behavioral repertoire of major workers is particularly limited, being frequently restricted to defense and/or food processing (WILSON, 1984; 2003). Indeed, when minors are experimentally removed from the colony, major workers take over many of their tasks, although with decreased efficiency (WILSON, 1984; MERTL; TRANIELLO, 2009). Major mandibles meet the demands to deal with the processing of hard food items through pressure, with their toothless masticatory margin spreading bite forces evenly around the food item. Seed consumption is considered an important aspect in the evolution of several myrmicine genera, such as *Pheidole, Pogonomyrmex*, and *Solenopsis* (FERSTER et al., 2006; MOREAU, 2008). However, the influence of granivory on morphological evolution, especially regarding the dimorphism in the *Pheidole* worker caste, is still poorly known (HOLLEY et al., 2016). Here, we demonstrate for the first time how ant mandible morphology can be tuned to deal with the mechanical demands of processing hard food items, such as seeds and arthropod cuticles, through the better performance of majors' mandibles in pressure biting conditions. Also, mandibles of *P. hetschkoi* majors show an even better performance in pressure bite than *P.* cf. *lucretii*, suggesting that majors of *P. hetschkoi* can deal better with harder food items than *P.* cf. *lucretii*. These results may lead to the possibility of food partitioning among

Pheidole coexisting species and agree with the habit of seed consumption by *P. hetschkoi*, which demands higher bite forces and consequently leads to higher stress levels on the mandibles.

In general, *Pheidole* minor mandibles show a more serrated and sharped *mm*, with well-developed teeth, whereas majors have mandibles with more blunted and broader *mm* (HUANG, 2012). However, the particular specimen of *P.* cf. *lucretii* included in our study showed high levels of teeth wear, allowing us to assess the consequences of teeth wear on bite loading. Teeth concentrate the forces generated by the masticatory muscles on smaller areas, with the potential to improve the initiation of fracture in the gripped object (CLISSOLD, 2007). The importance of teeth to task efficiency was demonstrated for leaf-cutting ants, where workers specialized to cut leaves switch to carrying them once their teeth are worn to a certain degree, reducing their cutting efficiency (SCHOFIELD et al., 2011). In *Pheidole*, minors perform a wide range of tasks in the colony (WILSON, 1984; 2003), but information on the role of teeth wear in minor task switching is scarce. Here we demonstrate the possible mechanical consequences of teeth wear in ant mandibles, comparing the relative amount of stress generated during masticatory margin strike simulations in *P. hetschkoi* and *P.* cf. *lucretii* minors. Our results indicated that *P.* cf. *lucretii* has relatively higher stresses than *P. hetschkoi*, mainly along its internal face of the mandible, which drives to higher stresses at the mandibular articulations with the head. Further studies in task allocation and mandible morphology in dimorphic ants species can address if teeth wear generates task switch, and biomechanical studies can reveal how teeth wear reduces task efficiency (SCHOFIELD et al., 2011). Also important is to understand if cuticle hardening by heavy metal bioaccumulation in the mandibles' masticatory margin (SCHOFIELD et al., 2002; BRITO et al., 2017) could help to mitigate the stress levels reaching the mandibular faces and articulations, as suggested for genital damage in bush crickets (MATSUMURA et al., 2020).

The morphological evolution of *Pheidole* might be strongly driven by differences in size (PIE; TRANIELLO, 2007), which tends to evolve at higher rates than shape (PIE; TSCHÁ, 2013; ECONOMO et al., 2015a; FRIEDMAN et al., 2019). More recently, studies applying geometric morphometrics approaches validated the prominence of size to explain the morphological disparity in the genus but also pointed to different evolutionary rates and levels of integration between head and mesosoma shape and size (FRIEDMAN et al., 2019; 2020). *Pheidole* morphological diversification seems to be very constrained (PIE; TRANIELLO, 2007), in contrast to their ecological disparity (ECONOMO et al., 2015a, 2015b), as reflected in the widespread distribution of the genus throughout most of the terrestrial ecosystems (ECONOMO et al., 2019). Field observations demonstrate that, despite the relative morphological resemblance in *Pheidole* species, they can show considerable ecological and behavioral diversity (MERTL et al., 2010; TSCHÁ; PIE, 2019). Here we demonstrate that even small morphological differences in mandible shape between species can lead to biomechanical specialization, mainly in majors food processing capacity, as suggested by descriptions of the mandibular morphology variation in *Pheidole* species (HUANG, 2012). This biomechanical specialization can expand the diet range of species and contribute to food partitioning (BLÜTHGEN et al., 2003; ROSUMEK, 2017; ROSUMEK et al., 2018), decreasing the degree of competition and allowing for species coexistence (BLÜTHGEN; FEELDAR, 2010).

Our results provide a biomechanical basis to understand how mandible morphological evolution can improve task specialization in polymorphic ants, and help develop a general understanding of form-function relationships in ant mandibles. Morphological polymorphism in the worker caste can expand the range of prey items that a species can handle, as demonstrated for some species of the army ant genus *Eciton* (POWELL; FRANKS, 2005; 2006). In the highly polymorphic genus *Cephalotes*, which is *Pheidole*'s sister lineage, some workers are specialized to plug the nest entrances with their heads to protect the colony against invasion (POWELL, 2008). In some fire ants such as *Solenopsis geminata* (Fabricius), the degree of worker polymorphism is associated with higher levels of division of labor, with major workers being specialized in seed milling (WILSON, 1978; FERSTER et al., 2006). Division of labor in leaf-cutting ants is associated with morphological distinctions among worker mandibles, as demonstrated for the polymorphic genus *Atta* (CAMARGO et al., 2016). In addition, refined morphological descriptions of *Pheidole* workers mandibles suggest that differences in the masticatory margin can improve task specialization (HUANG, 2012).

Therefore, the role of worker polymorphism for division of labor in ants is well established (WILLS et al., 2018), but by applying biomechanical approaches we can advance our understanding about the functional role of morphological disparity, as we demonstrated here for *Pheidole* workers. Ant polymorphic lineages are ideal models to investigate form-function relationships, and the morphological differentiation of mandibles should be studied in detail, given the importance of this structure to worker interactions with the environment. Future studies can contribute to our knowledge on the evolution of ant mandibles morphology by investigating the role of metal bioaccumulation

(HILLERTON; VINCENT, 1982; SCHOFIELD et al., 2003; POLIDORI et al., 2020) and the consequent cuticle hardening (SCHOFIELD et al., 2002; BRITO et al., 2017) in the mechanical response of mandibles to bite loading demands. Additionally, biomechanical approaches investigating the variation in bite force in morphologically polymorphic ant lineages can provide valuable information for the evolution of task specialization (HUANG, 2012), and understand how mandible morphology can be optimized to deal with powerful or fast movements (LARABEE et al., 2018).

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2.7 Tables and figures

Table 1. Size measurements of each worker and characteristics of each finite element mesh.

Specimen	Mesh superficial area (mm^2)	Element edge length (mm)	Number of elements	Voxel size (μm)	Mandible length (mm)	Head width (mm)
P. hetschkoi (major)	2.35	0.023	449488	5.30876	1.10	1.84
P. cf. *lucretii* (major)	1.011	0.023	278634	4.49981	0.85	1.18
P. hetschkoi (minor)	0.503	0.0035	881691	3.89985	0.65	0.80
P. cf. *lucretii* (minor)	0.25	0.0035	392790	4.04989	0.47	0.58

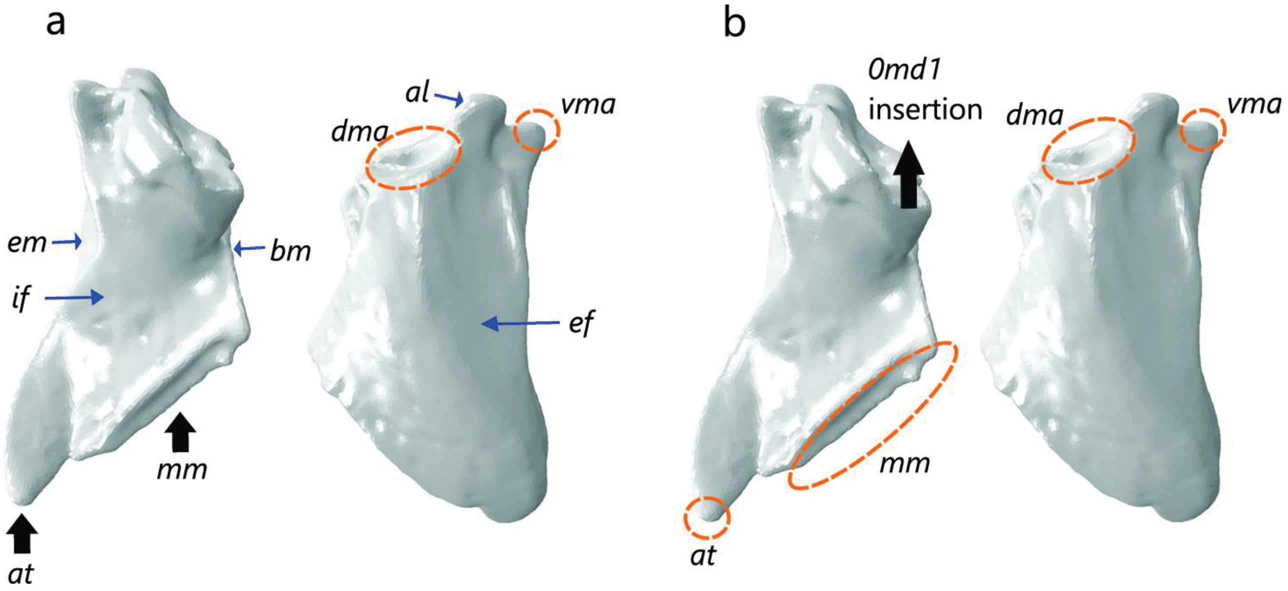

Fig. 1. Loaded and constrained regions in strike (a) and pressure (b) biting simulations. In (a) and (b), black arrows indicate the direction and region of load, and dashed lines enclose the constrained regions for each simulation. al: atala; at: apical tooth; dma: dorsal mandibular articulation; em: external margin; ef: external mandibular face; bm: basal margin; if: internal face of the mandible; 0md1: muscle *craniomandibularis internus*; mm: masticatory margin; vma: ventral mandibular articulation.

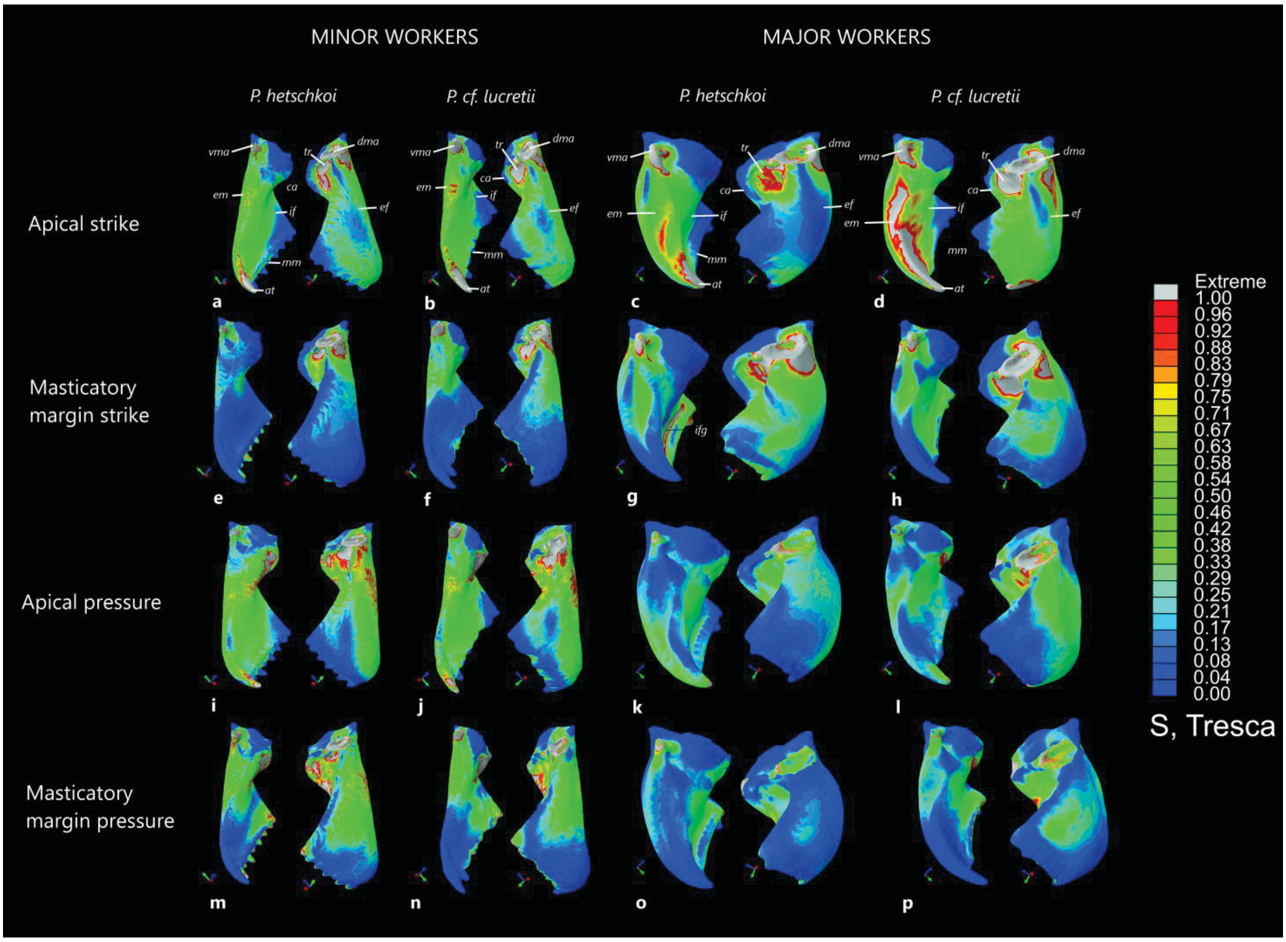

Fig. 2. Tresca stress results (rescaled to range between 0-1) for the four biting scenarios (rows), from minors and majors of both *Pheidole* species (columns). Each letter depicts a distinct simulation. Color represents a proportional value of stress in relation to the maximum value considered for each simulation, indicated as 1.00, and grey represents extremes values above the maximum considered. at: apical tooth; ca: canthellus; dma: dorsal mandibular articulation; ef: external mandibular face; em: external margin; if: internal face of the mandible; ifg: groove on the internal face of the mandible; mm: masticatory margin; tr: trulleum; vma: ventral mandibular articulation.

3. **MANUSCRIPT 2**

Head shape and bite loading demands in *Pheidole* ant workers

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Cristian L. Klunk, Alexandre Casadei-Ferreira and Marcio R. Pie conceived the ideas and designed methodology; Cristian L. Klunk collected the data; Cristian L. Klunk and Marco A. Argenta analyzed the data; Cristian L. Klunk and Marcio R. Pie led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

3.1 Abstract

Workers in the ant genus *Pheidole* show an extreme degree of morphological differentiation, with at least two distinct subcastes: minor workers are smaller and perform most of the colony tasks, whereas majors are larger, display disproportionately massive heads, and specialize in roles as defense and food processing. There is considerable interspecific variation in head shape within worker subcastes of *Pheidole*, which could affect how the stresses generated by the mandibular closing muscle contraction (0md1) spread throughout the head and influence bite force. To assess the role of head shape in stress patterns of *Pheidole* workers, we solve a set of Finite Element Analysis (FEA) while exploring variation in *Pheidole* worker head morphospace. We hypothesize that majors possess head shapes optimized for the generation of stronger bites. In addition, we expect that head shapes corresponding to the edges of morphological space in the genus would show mechanical limitations that could prevent further expanding the occupied morphospace. We vectorized *Pheidole* head shapes based on images of worker heads, considering species that represent mean shapes and the edges of the two main axes of each morphospace, for a total of five head shapes for each worker subcaste. We performed linear static FEA simulating the contraction of 0md1. Our results demonstrate that head shapes of majors are optimized to generate stronger bites given that stress generated on those shapes is distinctly directed towards the mandibles. Head shapes of minors tended to concentrate stresses around the mandibular articulations, with substantially lower and more diffuse stresses spreading throughout the head, indicating that such shapes are associated with weaker bites. Our results agree with the expectations regarding the main colony tasks performed by each worker subcaste, and we find some evidence of biomechanical limitations on extreme head shapes for majors and minors.

3.2 INTRODUCTION

In the genus *Pheidole*, workers split into two morphologically discrete subcastes, the minor and major workers (WILSON, 1953; 2003). Minors are small and slender, whereas majors are more robust and have disproportionately larger heads (PIE; TRANIELLO, 2007; LILLICO-OUACHOUR et al., 2018; RAJAKUMAR et al., 2018; FRIEDMAN et al., 2020). While minors are responsible for most of the colony's non-reproductive tasks, majors usually are associated with specialized responsibilities such as defense and food processing (WILSON, 1984; 2003; MERTL; TRANIELLO, 2009). Workers rely on their mandibles to execute most colony tasks, which involve behaviors such as biting, excavating, carrying, cutting, and fighting (WHEELER, 1910).

Size might be the primary driver of morphological differentiation between *Pheidole* worker subcastes (PIE; TRANIELLO, 2007), potentially evolving at higher rates than shape (PIE; TSCHÁ, 2013; ECONOMO et al., 2015; FRIEDMAN et al., 2019). However, there is evidence that the head shape of worker subcastes evolved more independently from each other than the mesosoma shape (FRIEDMAN et al., 2020). The specialized roles of majors in food processing suggest that diet can be a driver of *Pheidole* worker morphological evolution, mainly due to the necessity to process hard food items (HOLLEY et al., 2016; CASADEI-FERREIRA et al., 2021). Recent attempts suggest that the morphological variation between *Pheidole* species in head shape and size seems not to be related to differences in diet (HOLLEY et al., 2016; CASADEI-FERREIRA et al., 2021), but how the head shape is associated with the mechanical demands of bite according to the specific activities of each worker type still is an open question. Recent works suggest that even slight morphological modifications in *Pheidole* worker mandibles could lead to differences in bite performance between worker subcastes and species (HUANG, 2012; KLUNK et al., 2021), as also observed in other ants (LARABEE et al., 2018).

Given the prominence of mandibular closing muscles (0md1) in ant workers, we can expect that muscle contraction exerts significant mechanical demands on the worker's head capsule, with patterns of stress generated by the 0md1 contraction potentially varying according to head shape. We aimed to investigate the relationship between head shape and biomechanical performance by Finite Element Analysis (FEA) (KUPCZIK, 2008) in *Pheidole* worker plane head shapes. Although a simplistic perspective, the use of 2D data in biomechanical simulations proved to be effective as a first approximation to the mechanical demands of complex structures (MARCÉ-NOGUÉ et al., 2013), as demonstrated for the effects of biting behavior in crocodilians (PIERCE et al., 2008; 2009), theropods (RAYFIELD, 2005) and *Tyrannosaurus rex* (RAYFIELD, 2004) skulls, as well as bite loading in vertebrate jaws (DEAKING et al., 2022) and the mechanical consequences of borrowing on the trilobite cephalic region (ESTEVE et al., 2021).

Here we considered *Pheidole* species that represent the main variation in worker plane head shapes based on previously published morphospaces of major and minor *Pheidole* workers (CASADEI-FERREIRA et al., 2022). In doing so, we avoid a more subjective choice of taxa and maximize morphological variation (TSENG, 2021). Many of those species were included in the most recent phylogeny of the genus, showing a varied degree of phylogenetic relatedness (ECONOMO et al., 2015), representing lineages from the Neotropics, Australasia, Africa, and Madagascar. From the ten *Pheidole* species here considered, six were present in that phylogeny, and diverged very anciently in *Pheidole*'s diversification history (ECONOMO et al., 2015). We hypothesize that the mean plane head shape of *Pheidole* major and minor workers will display substantial differences in stress patterns, with majors showing patterns associated with the capacity to deal with stronger bite forces than minors. Also, we expect that the plane shapes located on the morphospaces' extremes will show distinct stress patterns from the mean plane shapes, with signs of mechanical limitations that prevent its higher frequency on current *Pheidole* lineages. Given that the amount of head cuticle that shows any sculpturing pattern varies intra and interspecifically among *Pheidole* species (personal observations), we also tested if such variation is associated with stress patterns generated by the 0md1 contraction, hypothesizing that majors have an increased area of the head dorsal wall covered with sculptures than minors.

3.3 MATERIALS AND METHODS

3.3.1 *Head shapes*

We chose *Pheidole* species with particularly extreme morphologies by exploring the morphospace inferred by Casadei-Ferreira et al. (2022) using 2D geometric morphometrics data. The plane axis considered by Casadei-Ferreira et al. (2022) contemplates variation in head height and width, two of the main characteristics associated with the morphological variation among *Pheidole* worker heads (CASADEI-FERREIRA et al. 2021). We selected *Pheidole* species close to the edges of the first two

PCA axes and the mean shape for each subcaste (CASADEI-FERREIRA et al., 2022), totaling ten species (five for each subcaste, Table 1). We vectorized head shapes using Inkscape ® based on images of specimens available on AntWeb (2021) and exported those vectors as OBJ files using Blender 2.83 ®. With Fusion 360 (AUTODESK), SAT geometries were generated using the OBJ files and imported into the finite element solver Abaqus 6 (Dassault Systèmes).

3.3.2 *Finite element analysis*

We apply unitary and constant thickness to head plane models to define plane stress analysis. Such a procedure considers that the modeled structure has two main dimensions, and the stresses in the third dimension are negligible. The finite element meshes were designed with plane triangular quadratic elements (CPS6M). We define the mesh density after a mesh convergence procedure of three plane head shapes (i.e., *P*. *flavens*, *P*. *grallatrix*, and *P*. *obtusospinosa*), which are good representatives of the morphological variation of the plane head shapes here considered. We defined more simplistic loading and boundary conditions to perform the mesh convergence tests and analyzed the variation in Tresca equivalent nodal stress values with changes in mesh density. Once the error between the current and last mesh densities in nodal Tresca stress achieved less than 2% in three different nodes, we chose the coarser mesh of the converged pair to represent the final mesh density. Mesh convergence was achieved at the same density in all three plane head models (Table 2), so we applied the defined mesh density to all plane head representations (Table 1). We determined the material properties according to data available in the literature. Therefore, we designated Young's modulus as 2.75 GPa (BRITO et al., 2017) and the Poisson ratio as 0.3 (LARABEE et al., 2018; ZHANG et al., 2020; KLUNK et al., 2021; WANG et al., 2022). We determined the cuticle as an isotropic and linear elastic material and applied the same homogeneous material properties for each head plane model.

To simulate the loads generated by the contraction of mandibular closing muscles, we applied normal loads on the nodes of each head side (Fig.1), approximating the pennation angle of 0md1 usually observed in ant workers (PAUL, 2001; PAUL; GRONENBERG, 2002; LILLICO-OUACHOUR et al., 2018; RICHTER et al., 2019; 2020; 2021; BOUDINOT et al., 2021; PÜFFEL et al., 2021) that maximizes force generation, as suggested for leaf-cutting ants (PÜFFEL et al., 2021). We applied a 1 N load on each head side to the model with the largest surface area and normalized the load of the remaining models according to the difference in surface area from the reference model (Table 1) (MARCÉ-NOGUÉ et al., 2013). We fixed nodal displacement to zero in all directions in the corners of the head base. This procedure approximates the positioning of the mandibles to simulate their reaction forces during a bite. We fixed the same number of nodes in each head side and model. We performed one linear static simulation for each head shape.

To visualize the resulting stress patterns, we employed tensor plots of principal stresses (Fig.2). Principal stresses are normal stresses that occur at plane orientations where sheer stress is zero, represented by minimum and maximum principal stresses (HIBBELER, 2017). Arrows indicate the direction (positive or negative along the x and y axes), relative normalized magnitude (arrow size), and stress type (compression or tensile, according to the orientation of the arrowheads). Green arrows depict the minimum principal stresses and represent here essentially compressive stresses (arrowheads pointing inwards) (Fig.2). Blue arrows, otherwise, represent the maximum principal stresses, which range here from compressive to tensile stresses (arrowheads pointing outwards) (Fig.2). To have a more comprehensive visualization of the distribution of stress levels along *Pheidole* worker plane heads, we also employed color maps depicting stress variation in normalized magnitude, based on a failure criterion. We can represent stress values from FEA under several stress variables, being the better choices related to the structure material behavior. In a state of plane stress, as simulated here, any element is subjected to two normal and one shear stress (HIBELLER, 2017). To reliably consider the material strength and its resistance to failure, such combined sources of stress are reduced to a single stress component by applying a stress transformation that follows a specific failure criterion. Here we considered the Tresca failure criterion, which is more conservative (ÖZKAYA et al., 2017). Tresca failure criterion assumes that material failure happens due to shear stress, and the direction of principal stresses observed on our tensor plots (Fig.2) justifies the adoption of this criterion. We scaled the stress range of each simulation based on the maximum stress value of a reference model to improve visualization and allow comparison between species. Therefore, the non-normalized stress values of each simulation are meaningless in those plots, and we interpreted the qualitative differences between simulations as representing proportional differences in stress distribution.

3.3.3 *Statistical analyses*

We applied the intervals method (MARCÉ-NOGUÉ et al., 2017) to evaluate how plane head shapes differ in the area covered by distinct ranges of stress values. This method defines comparable stress intervals from FEA results and calculates the area covered by each stress interval based on the sum of the element area. From a predefined upper threshold of stress, which determines the highest stress interval in the dataset and is a reference for the generation of the remaining stress intervals, the method can generate any number of stress intervals, sum up the area occupied by those intervals on each model, and calculate the proportion of area covered by each interval for all models. These area proportions are new variables that can be used in further statistical tests or ordination analyses, such as Principal Component Analyses (PCA) (MARCÉ-NOGUÉ et al., 2017).

To apply this method, we extracted values of stress and area from the elements of each plane head model and removed the elements representing the 2% highest stress values for each simulation since they usually represent artificially high values (MARCÉ-NOGUÉ et al., 2016; 2017). We transformed the stress values to their natural logarithm to avoid the influence of outliers. We defined the upper threshold value as 0.69, to characterize the highest stress interval with only 2% of the highest stress values from all simulations (MARCÉ-NOGUÉ et al., 2017). We generated datasets with distinct intervals (5, 15, 25, 50, and 75) and performed a PCA with each dataset to define the ideal number of stress intervals. We considered the scores of PC1 and PC2 of each dataset as variables in linear regressions with the scores of equivalent PCs of the next stress interval (e.g., $PC1_{5intervals} \sim PC1_{15intervals}$), considering the coefficient of determination (R^2) to analyze the convergence of PC scores (MARCÉ-NOGUÉ et al., 2017). When R^2 ceased to increase, the number of intervals of the dataset where R^2 converged was considered the final number of intervals (MARCÉ-NOGUÉ et al., 2017). Convergence occurred within 15 intervals. Therefore, we conducted the PCA with this dataset to investigate how plane head shapes differ in the proportion of head area covered by the distinct intervals of stress. We performed all statistical analyses in R version 4.1.3 (R CORE TEAM, 2022). We also tested if the plane head shapes vary in the magnitude of Tresca equivalent stress values through a Kruskall-Wallis test and post hoc Dunn tests with Bonferroni corrections for repeated tests.

Finally, we tested whether *Pheidole* workers differ in the sculpturing pattern of their heads according to the distinct stress distributions observed in the tensor and color plots. We defined four categories of cuticle sculpturing: category 0 represents species with an overall smooth head cuticle, showing some sculpture around the antennal insertion; category 1 represents species with some sculpturing around the regions of mandibular articulation; category 2 denotes species with additional sculpture spreading toward the head lateral margins and; category 3 stands for species whose most head dorsal cuticle is sculptured. We classify sculpturing patterns of 143 *Pheidole* species considered in Casadei-Ferreira et al. (2022) for the head shape morphospace of *Pheidole* species. We obtained worker full-face pictures from an online repository (ANTWEB, 2021). Whenever more than one picture for each worker type was available, and there was variation in sculpture patterning, we classified the worker according to the highest category detected, prioritizing the patterns found on type specimens. Then, we tested if *Pheidole* workers differ in head sculpturing pattern among those four categories through a chi-squared test, with a post hoc test with Bonferroni correction to test for pairwise differences between sculpture categories, using the R package chisq.posthoc.test (EBBERT, 2019). We used the R package dplyr version 1.0.9 to manipulate the data (WICKHAM et al., 2022), FactoMineR version 2.4 (LÊ et al., 2008), and factoextra version 1.0.7.999 (KASSAMBARA; MUNDT, 2020) to perform the PCA, and ggplot2 version 3.3.5 (WICKHAM, 2016), ggpubr version 0.4.0 (KASSAMBARA, 2020), viridis version 0.6.2 (GARNIER, 2021), tidyverse version 1.3.1 (WICKHAM et al., 2019) and hrbrthemes version 0.8.0 (RUDIS, 2020) to generate plots.

3.4 RESULTS

3.4.1 *Tensor plots*

The plane head shape of majors and minors showed differences in stress patterns. *Pheidole flavens*, which approximates the mean shape of majors, showed proportionally higher levels of stress spreading throughout a larger area of the head, mainly along its lateral margins following the direction of muscle contraction (Fig.2). On the other hand, the mean shape of minors, represented by *P. obtusospinosa*, showed a concentration of stress around the regions of mandibular articulation, but proportionally lower stress levels distributed throughout the remaining of the head (Fig.2). Among majors, plane head shape varies more subtly in stress patterns across the morphospace than in minors. *Pheidole absurda* (PC1max) and *P*. epem121 (PC2min) showed a stress pattern similar to that of the mean shape, whereas *P*. *biconstricta* (PC1min) and *P*. *pallidula* (PC2max) exhibited slightly reduced stress levels along the head, especially on the lateral margins (Fig.2). Minors have a higher morphological variation along the morphospace, although their stress patterns are also similar in general, except that *P*. *hercules* (PC2max) showed a considerable amount of stresses along the posterior margin of the head, and *P*. *casta* showed proportionally higher stresses along the lateral margins of the head (Fig.2). In a thinner analysis considering tensor plots, *P*. *hercules* draws attention due to a reduced concentration of stress on the head center than the remaining shapes (Fig.2), which is possibly related to its wider head, leading to significant dissipation before stresses achieve the central area of the head. In *P*. *grallatrix* (PC1min), which has a narrower head, an opposite pattern was observed, with a denser concentration of stress on the central head region, especially of compressive stresses along the x-axis (lateral compression) (Fig.2). *Pheidole casta* (PC2min) and *P*. *kohli* (PC1max) showed similar stress patterns, having in general higher stress levels than *P*. *hercules* and *P*. *obtusospinosa*, and proportionally higher levels of vertical compressive stresses along the lateral margins than *P*. *grallatrix*, although at substantially lower levels than what happens in majors (Fig.2).

3.4.2 *Intervals method*

To evaluate quantitatively the proportion of head area covered with different stress intervals, we used a PCA whose input variables were the proportional amount of area covered by each of the 15 intervals of stress. The first two components explained 85.54% of the variance and were considered here for further discussion. PC1 explained 60.37% of the variance and split species with proportionally larger areas of the head covered by intervals of intermediate stress levels (i.e., *P*. *absurda* and *P*. epem121) from species with a larger area covered by the lowest stress interval (i.e., *P*. *kohli*) (Table 3). Interestingly, the positive range of PC1 is occupied predominantly by plane head shapes of majors, except for the presence of P. casta. In contrast, the PC1 negative spectrum depicts mostly minor worker plane heads, except for *P*. *biconstricta*. The head shape of *P*. *flavens* lies on the origin (Fig.3), suggesting a more homogeneous distribution of stress intervals. PC2 explained 25.17% of the variance and is positively associated with intervals 10-11 (Table

3). This component mainly separates *P*. *pallidula* and *P*. *grallatrix* from the remaining species in its positive range (Fig.3).

3.4.3 *Tresca equivalent stress values*

Non-normalized stress levels based on Tresca failure criteria differed significantly between *Pheidole* plane head shapes after removing from each model their 2% highest stress values (Kruskall-Wallis c^2_9 = 10547; $p < 0.001$). *Pheidole absurda* showed the highest mean stress value among all shapes (0.78 N/mm^2), whereas *P*. *kohli* showed the lowest mean stress (0.65 N/mm^2; Table 1; Fig.4). Only two species pairs showed no difference in mean stress levels after *post hoc* Dunn tests with Bonferroni corrections for repeated tests, which were *P*. *flavens* versus *P*. *hercules* and *P*. *biconstricta* versus *P*. *pallidula* (Table 4). Although statistically different, the mean non-normalized stress values of *P*. *flavens* (0.68) was only slightly lower than for *P*. *obtusospinosa* (0.69) (Table 1; Fig.4).

3.4.4 *Cuticle sculpturing pattern*

Pheidole workers differed in the amount of sculpture covering the dorsal head cuticle (c^2 = 46.83, df = 3, p = 3.778e-10). Specifically, minors of *Pheidole* species tended to have sculpturing patterns of categories 0 and 1 (small regions of the head sculptured), and majors patterns of category 3 (almost the complete dorsal area of the head covered with some sculpture), whereas there was no difference regarding category 2 (Table 5; Fig.5).

3.5 DISCUSSION

Our FEA simulations demonstrated that plane head shape variation affects stress patterns due to 0md1 contraction. Tensor plots exposed substantial distinctions in stress patterns between the most common worker plane head shapes (i.e., the mean shapes). The head shape of *P*. *flavens* showed a propensity to dissipate stresses along a larger area of the head than the head shape of *P*. *obtusospinosa*, where stresses tended to concentrate more around the regions of mandibular articulation. Interestingly, such head shapes showed only a slight difference in the mean non-normalized stress value, with *P*.

obtusospinosa having a slightly higher value than *P*. *flavens*. Regarding the PCA based on the intervals method, the mean plane head shapes do not represent the most distant pair on PC1, with the head shape of *P*. *flavens* showing a more homogeneous distribution of stress intervals than *P*. *obtusospinosa*, which showed a larger area of the head covered with intervals of low stress. On PC2, their separation is more evident, with *P*. *flavens* showing a larger area of the head covered with intervals of intermediate stress levels when compared to *P*. *obtusospinosa*, which showed a slightly higher amount of head area covered by the highest stress interval, that is located around the regions of mandibular articulation.

Most major heads have a higher coverage of high-stress intervals than minor worker heads, agreeing with the patterns observed on tensor plots and colour maps, which suggest a more favorable stress dissipation in major worker plane head shapes. The plane head shapes of *P*. epem121 and *P*. *absurda* are particularly distinct because they depart even more from the remaining plane shapes regarding the percentage of area covered with intermediate toward high-stress values. These shapes also showed higher mean values of Tresca equivalent stresses. *Pheidole absurda* and *P*. epem121 represent plane head shapes located in more isolated regions of their morphospace (CASADEI-FERREIRA et al., 2022), which means that few *Pheidole* species explored such head contours, drawing attention to the possibility of biomechanical limitations related to high-stress generation on these head shapes that prevent their widespread among current lineages. On the other end of PC1, the plane head shape of *P*. *kohli* departs from the remaining head shapes by showing a larger area covered by the interval of lowest stress, reinforcing its tendency, observed on the tensor plot, to heavily concentrate stresses around the regions of mandibular articulation. This mechanical behavior may help to explain why the plane head shape of *P*. *kohli* is poorly explored in current *Pheidole* lineages (CASADEI-FERREIRA et al., 2022).

Head shape evolution is crucially associated with insect bite forces (BLANKE et al., 2017a; 2018). In general, there is a strong correlation between head width and bite force capacity in insects (BLANKE, 2019; RÜHR et al., 2022) since the generation of forceful bites demands an increase in mandibular closing muscle volume, which in turn requires wider heads to accommodate such larger muscles (PAUL, 2001; PAUL; GRONENBERG, 2002; PÜFFEL et al., 2021; 2023a). In ants, 0md1 occupies a significant volume of the worker head (PAUL; GRONENBERG, 2002; KHALIFE et al., 2018; LILLICO-OUACHOUR et al., 2018; RICHTER et al., 2019; 2020; 2021; BOUDINOT et al., 2021). Our results suggest that the plane head shape of majors and minors partially reflects the mechanical demands of bite associated with their main colony tasks. *Pheidole* majors typically have broader and heart-shaped heads that dissipate the stresses generated by the contraction of 0md1 along a wider plane head area, avoiding stress concentration and hence improving its capacity to deal with stronger bites. Minors, otherwise, usually have narrower and round-shaped heads that show more constrained stress spread, with a tendency to concentrate stresses on the regions of mandibular articulation. Associations between head shape and bite force in ant workers are not novel. Several authors already suggested that broader and heart-shaped heads can harbor a proportionally larger volume of muscle fibers than elongated heads, consequently generating stronger bites, whereas prolonged head shapes tend to be associated with a distribution of muscle fibers that benefit faster contractions (PAUL, 2001; PAUL; GRONENBERG, 2002; KHALIFE et al., 2018; PÜFFEL et al., 2021; RICHTER et al., 2023). The importance of bite force in *Pheidole* majors is clearly illustrated by the fact that 0md1 increases disproportionately in relation to minors, at the expense of other tissues of the worker’s head, such as the central nervous system (LILLICO-OUACHOUR et al., 2018). Our results, however, highlight that the morphological distinctions between head shapes go beyond their differences in the capacity to store muscle fibers and rely on idiosyncrasies in the responses to the mechanical demands of bite loading, as also suggested for *Melissotarsus* Emery 1877 ants (KHALIFE et al., 2018).

Broadening of the head is characteristic of some specialized ant worker subcastes, as happens in *Pheidole* (PIE; TRANIELLO, 2007; PIE; TSCHÁ, 2013; LILLICO-OUACHOUR et al., 2018), *Atta* (PÜFFEL et al., 2021), *Cephalotes* (POWELL, 2008), *Solenopsis* and *Pogonomyrmex* (FERSTER et al., 2006), among others. Morphological specialization in the ant worker caste is usually associated with some degree of task specialization (OSTER; WILSON, 1978; WILLS et al., 2018). In *Pheidole*, the division of labor strongly correlates with the morphological differences between worker subcastes. Majors are mainly recruited for food processing and defense (WILSON, 1984; MERTL; TRANIELLO, 2009), behaviors that demand stronger and long-standing bites, like crushing food items. Minors lead the remaining non-reproductive colony tasks, such as brood care, foraging, and colony maintenance (WILSON, 1984; MERTL; TRANIELLO, 2009), which demand a more generalist use of the mandibles, including the generation of faster, subtler and more repeatedly mandibular movements.

Besides the main morphological differences between *Pheidole* workers, there is substantial interspecific variation in worker morphology that potentially reflects ecological specializations. The need to capture and process food can considerably affect morphological evolution. In ants, such demands will reflect mainly on the morphological evolution of the worker head and mandibles (POWELL; FRANKS, 2005; 2006; OHKAWARA et al., 2017; BARDEN et al., 2020; BOOHER et al., 2021). In *Pheidole*, the fact that several lineages add seeds to their diets (MOREAU, 2008; ROSUMEK, 2017) leads to the hypothesis that seed consumption could have been a significant evolutionary pressure towards the evolution of major workers, and to explain the interspecific morphological diversity of the genus (MOREAU, 2008). However, investigations so far did not reveal a meaningful relationship between the consumption of seeds and head size (HOLLEY et al., 2016) and shape (CASADEI-FERREIRA et al., 2021). Our results showed that plane head shapes of majors did not differ substantially in stress patterns. Although *Pheidole absurda* is the only species here considered known to consume seeds, it did not show a stress pattern considerably distinct that could suggest a better capacity to withstand stronger bites than the remaining head shapes. However, it showed the higher mean Tresca equivalent stress value among all plane head shapes, indicating that such a plane shape is subjected to relatively higher reaction forces from the mandibles. Together, those results agree with the fact that regardless of the consumption or not of seeds, majors generally are faced with tasks that demand stronger bites (WILSON, 1984; MERTL; TRANIELLO, 2009), as defense or even the processing of other hard food items, like arthropod exoskeletons. Therefore, the ecological demands associated with *Pheidole* majors seem to result in a morphological convergence regarding their head plane shape. Minors, otherwise, are faced with a more complex and generalist set of colony tasks (WILSON, 1984; MERTL; TRANIELLO, 2009), being submitted to a more diverse array of evolutionary pressures, potentially leading to the evolution of head shapes that depart more from the general stress pattern of the standard minor plane head shape, as observed on the tensor plots. Current evidence suggests that the geographic distribution of *Pheidole* species is strongly associated with worker head shape evolution (CASADEI-FERREIRA et al., 2022). In contrast, nesting habit seems to have the potential to limit the variation of major workers' head size (MERTL et al., 2010).

Majors of *Pheidole* showed an increased area of the head dorsal cuticle covered with some sculpturing pattern, which agrees with our hypothesis that majors have, in general, a sculpturing pattern that follows the distribution of stresses observed in their plane head shapes. Increased stress levels could require some cuticular reinforcement for such mechanical demands. More sculptured cuticles tend to be thicker (BUXTON et al., 2021), but even cuticles with the same thickness could potentially differ in their mechanical response when covered with distinct levels of surface roughness (HELLENBRAND, 2022). There is evidence that predatory ants, which may need to withstand higher mechanical demands to capture their prey, are more sculptured than non-predatory species (GIBB et al., 2015). However, cuticular sculpturing patterns can be associated with several distinct functions. The occurrence of sculptures is highly variable along the ant phylogeny (HELLENBRAND, 2022), so more effort is needed to investigate the role of cuticular sculpturing and mechanical resistance.

Our results reinforce the idea that evolutionary pressures in the morphological evolution of *Pheidole* workers seem to act independently between worker types (PIE; TRANIELLO, 2007; FRIEDMAN et al., 2020) due to distinct mechanical demands associated with their differential roles in the colony. *Pheidole* evolution has been investigated extensively, yet many aspects of its morphological diversity and worker dimorphism remain unexplored. Here we demonstrated that differences between the plane head shape of major and minor workers have profound implications for the stress patterns generated by the contraction of the mandibular closing muscles, agreeing with the suggestion that head shapes in *Pheidole* workers evolved more independently than other body regions (FRIEDMAN et al., 2020). Also, the plane head shape of majors differs less interspecifically than those of minors in stress patterns, suggesting that task specialization between *Pheidole* workers leads to a degree of convergence in head shape, especially leading to the evolution of broader and more heart-shaped heads in majors.

According to our results, the plane head shapes of majors generally have a better capacity to dissipate stresses along the head than minors, which tend to concentrate stresses on a small head area. Since no differences in cuticle thickness were being considered under our approach, which could justify the occurrence of stress concentration in thicker cuticle regions, such distinctions in stress patterns suggest that plane head shapes of majors can withstand stronger bites by dissipating stresses along a larger area of the head. Supporting this mechanism, we showed that the head shapes of majors usually have a considerably more sculptured dorsal surface, which increases the thickness of the cuticle and hence could improve the capacity to deal with higher stresses. Further studies are required to investigate in more detail the role of *Pheidole* worker head shape in bite mechanical performance, especially considering the three-dimensional morphology of this structure. This way, relevant aspects of the head morphology can be addressed, such as the role of the endoskeleton (BLANKE et al., 2018; KUBOTA et al., 2019; RICHTER et al., 2019; 2020; 2021; BOUDINOT et al., 2021) and cuticle thickness on stress distribution. Also, the influence of cuticular sculpturing on the mechanical response to loading remains to be further tested (BUXTON et al., 2021; HELLENBRAND, 2022). Despite being a more simplistic approach, applying FEA on plane structures proved to be a valuable tool to reveal differences in stress patterns between the head shapes of *Pheidole* workers. We suggest a more widespread application of this approach in other animal lineages for instances where 3D structures are too complex to allow the simultaneous investigation of many species (RAYFIELD et al., 2004; 2005; PIERCE et al., 2008; 2009; FLETCHER et al., 2010; ESTEVE et al., 2021).

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3.7. Tables and figures

Table 1. Main characteristics of the simulated head shape models of *Pheidole* workers, including species, subcaste type, position in the morphospace, undeformed mesh area, number of elements, load magnitude and mean Tresca equivalent stress value after simulation.

Species	Subcaste	Morphospace position	Mesh area (mm^2)	Number of mesh elements	Applied load (N)[1]	MTESV (N/mm^2)[2]
Pheidole absurda	Major	PC1 max	3.510	82981	1.00	0.78
Pheidole biconstricta	Major	PC1 min	1.710	82700	0.70	0.67
Pheidole pallidula	Major	PC2 max	1.410	80750	0.63	0.67
Pheidole epem121	Major	PC2 min	1.070	81912	0.55	0.71
Pheidole flavens	Major	Mean	0.665	81745	0.44	0.68
Pheidole kohli	Minor	PC1 max	0.951	85269	0.52	0.65
Pheidole grallatrix	Minor	PC1 min	0.225	82408	0.25	0.71
Pheidole hercules	Minor	PC2 max	0.556	82363	0.40	0.67
Pheidole casta	Minor	PC2 min	0.141	84075	0.20	0.69
Pheidole obtusospinosa	Minor	Mean	0.337	82784	0.31	0.69

[1] Load applied at each head side.

[2] Mean Tresca Equivalent Stress Value

Table 2. Details of the mesh convergence tests to define the mesh density of plane head models. Node refers to the number of the node where Tresca values were collected. X and Y coordinates depict the location of the node in the mesh along the X and Y axis of orientation. Set represents the head region of each node (A = on the center of the head; B = near to left mandibular articulation; C = near to right mandibular articulation). Mesh codes for the mesh density (1 = mesh density as applied on the final FEA; 2 = the coarser mesh density; 3 = the refined mesh density). Model identifies the *Pheidole* species. N nodes and N elements depict the respective number of nodes and elements of the correspondent mesh. Tresca represents the stress value of each node based on Tresca failure criteria. % error is the variable considered to define mesh convergence, and is the result of the following equation: (previous - current mesh) / previous mesh x 100. We considered that convergence was achieved when the % error was less than 2% for all nodes analyzed, and the coarser mesh in the convergent pair was considered as the definitive mesh density.

Node	X coordinate	Y coordinate	Set	Mesh	Model	N nodes	N elements	Tresca	Error(%)
38650	0.006466	0.178906	A	2	flavens	81505	40488	2.50291	X
66807	-2.82E-01	-1.06E-01	B	2	flavens	81505	40488	2.39421	X
77019	2.79E-01	-9.84E-02	C	2	flavens	81505	40488	2.16312	X
5384	0.007301	0.17675	A	1	flavens	164238	81745	2.47437	1.14
21627	-2.81E-01	-1.07E-01	B	1	flavens	164238	81745	2.43224	1.59
21777	2.78E-01	-9.88E-02	C	1	flavens	164238	81745	2.17422	1.11
239695	7.55E-03	1.76E-01	A	3	flavens	328073	163504	2.45938	0.61
34118	-2.82E-01	-1.07E-01	B	3	flavens	328073	163504	2.4247	0.31
290906	2.79E-01	-9.89E-02	C	3	flavens	328073	163504	2.19084	0.76
79525	2.73E-01	7.42E-02	A	2	obtusospinosa	83533	41504	2.02238	X

68221	6.99E-02	-6.31E-02	B	2	obtusospinosa	83533	41504	2.98106	X
19984	4.46E-01	-6.96E-02	C	2	obtusospinosa	83533	41504	2.94179	X
40586	0.272442	7.49E-02	A	1	obtusospinosa	166311	82784	2.01862	0.19
2836	6.91E-02	-6.28E-02	B	1	obtusospinosa	166311	82784	2.99713	0.54
34077	4.46E-01	-7.01E-02	C	1	obtusospinosa	166311	82784	2.95327	0.39
229159	2.73E-01	7.51E-02	A	3	obtusospinosa	326340	162645	2.00945	0.45
141380	6.97E-02	-6.27E-02	B	3	obtusospinosa	326340	162645	2.98554	0.39
69249	4.46E-01	-6.99E-02	C	3	obtusospinosa	326340	162645	2.96346	0.35
73486	1.285383	1.84E-01	A	2	grallatrix	82768	41115	2.95163	X
70125	1.099536	-2.19E-02	B	2	grallatrix	82768	41115	3.89791	X
74260	1.410021	-2.27E-02	C	2	grallatrix	82768	41115	3.60143	X
34910	1.272866	0.183519	A	1	grallatrix	165581	82408	2.91798	1.14
15149	1.096732	-2.24E-02	B	1	grallatrix	165581	82408	3.9874	2.3
20364	1.414002	-2.24E-02	C	1	grallatrix	165581	82408	3.66326	1.72
12680	1.271491	1.84E-01	A	3	grallatrix	329473	164198	2.91059	0.25
27178	1.096557	-2.22E-02	B	3	grallatrix	329473	164198	4.01536	0.7
138209	1.414844	-2.24E-02	C	3	grallatrix	329473	164198	3.6664	0.09

Table 3. Correlations between stress intervals and the two first dimensions of the PCA (Fig.3 on the main text).

Stress interval	**Dim.1**	**Dim.2**
V1	-0.966	0.0818
V2	0.864	-0.314
V3	0.942	-0.257
V4	0.953	-0.140
V5	0.959	-0.126
V6	0.979	0.060
V7	0.957	0.202
V8	0.866	0.437
V9	0.557	0.783
V10	0.078	0.925
V11	0.247	0.949
V12	0.695	0.380
V13	0.768	-0.167
V14	0.634	-0.598
V15	0.432	-0.6583

Table 4. Results of post-hoc dunn tests with bonferroni corrections for repeated tests for the difference in stress magnitude between each pair of *Pheidole* species simulations.

Comparisons	chi2	Z	P.adjusted
P.absurda - P.biconstricta	10546.8878175826	73.292504921378	0
P.absurda - P.casta	10546.8878175826	59.6311714396493	0
P.biconstricta - P.casta	10546.8878175826	-13.95134429	6.95E-43
P.absurda - P.epem121	10546.8878175826	48.7965974457227	0
P.biconstricta - P.epem121	10546.8878175826	-24.29932989	4.48E-129
P.casta - P.epem121	10546.8878175826	-10.48117174	2.37E-24
P.absurda - P.flavens	10546.8878175826	68.4330846901254	0
P.biconstricta - P.flavens	10546.8878175826	-4.642001503	7.76E-05
P.casta - P.flavens	10546.8878175826	9.24945792630671	5.08E-19
P.epem121 - P.flavens	10546.8878175826	19.598067641973	3.61E-84
P.absurda - P.grallatrix	10546.8878175826	38.162799600545	0
P.biconstricta - P.grallatrix	10546.8878175826	-35.03496153	1.49E-267
P.casta - P.grallatrix	10546.8878175826	-21.23991646	9.19E-99
P.epem121 - P.grallatrix	10546.8878175826	-10.67319676	3.06E-25
P.flavens - P.grallatrix	10546.8878175826	-30.29534442	2.97E-200
P.absurda - P.hercules	10546.8878175826	67.5896737390242	0
P.biconstricta - P.hercules	10546.8878175826	-5.622929302	4.22E-07
P.casta - P.hercules	10546.887817582	8.2909405044041	2.53E-15

	6	9	
P.epem121 - P.hercules	10546.8878175826	18.6651063748704	2.14E-76
P.flavens - P.hercules	10546.8878175826	-0.96933242	1
P.grallatrix - P.hercules	10546.8878175826	29.3812114366075	2.15E-188
P.absurda - P.kohli	10546.8878175826	86.3136460846668	0
P.biconstricta - P.kohli	10546.8878175826	12.4512596757759	3.10E-34
P.casta - P.kohli	10546.8878175826	26.561740402069	4.23E-154
P.epem121 - P.kohli	10546.8878175826	36.9044431888899	8.82E-297
P.flavens - P.kohli	10546.8878175826	17.0916461741207	3.85E-64
P.grallatrix - P.kohli	10546.8878175826	47.7413660052331	0
P.hercules - P.kohli	10546.8878175826	18.1039865672053	6.67E-72
P.absurda - P.obtusospinosa	10546.8878175826	52.718677127619	0
P.biconstricta - P.obtusospinosa	10546.8878175826	-20.57496099	1.04E-92
P.casta - P.obtusospinosa	10546.8878175826	-6.704715407	4.54E-10
P.epem121 - P.obtusospinosa	10546.8878175826	3.77986098251649	0.00353060876753995
P.flavens - P.obtusospinosa	10546.8878175826	-15.87192569	2.23E-55
P.grallatrix - P.obtusospinosa	10546.8878175826	14.4871056938385	3.29E-46
P.hercules - P.obtusospinosa	10546.8878175826	-14.92954127	4.76E-49
P.kohli - P.obtusospinosa	10546.8878175826	-33.18620325	3.83E-240
P.absurda - P.pallidula	10546.8878175826	71.6709120721437	0
P.biconstricta - P.pallidula	10546.887817582	-1.181357841	1

	6		
P.casta - P.pallidula	10546.8878175826	12.6810336332328	1.69E-35
P.epem121 - P.pallidula	10546.8878175826	22.9760790683341	1.82E-115
P.flavens - P.pallidula	10546.8878175826	3.43642518954311	0.0132625150804262
P.grallatrix - P.pallidula	10546.8878175826	33.6453927906373	8.19E-247
P.hercules - P.pallidula	10546.8878175826	4.40919243442697	0.000233452798766626
P.kohli - P.pallidula	10546.8878175826	-13.56588169	1.44E-40
P.obtusospinosa - P.pallidula	10546.8878175826	19.2701458888922	2.15E-81

Table 5. Results of a post-hoc test based on the Bonferroni correction for difference in the count of rugosity pattern between major and minor workers of *Pheidole*.

Rugosity pattern	**Variable**	**Major**	**Minor**
0	N species	1	20
0	Residuals	-4.29	4.29
0	p values	0.00014	0.00014
1	N species	23	57
1	Residuals	-4.46	4.46
1	p values	6.50E-05	6.50E-05
2	N species	18	10
2	Residuals	1.61	-1.61
2	p values	0.852286	0.852286
3	N species	97	53
3	Residuals	5.35	-5.35
3	p values	1.00E-06	1.00E-06

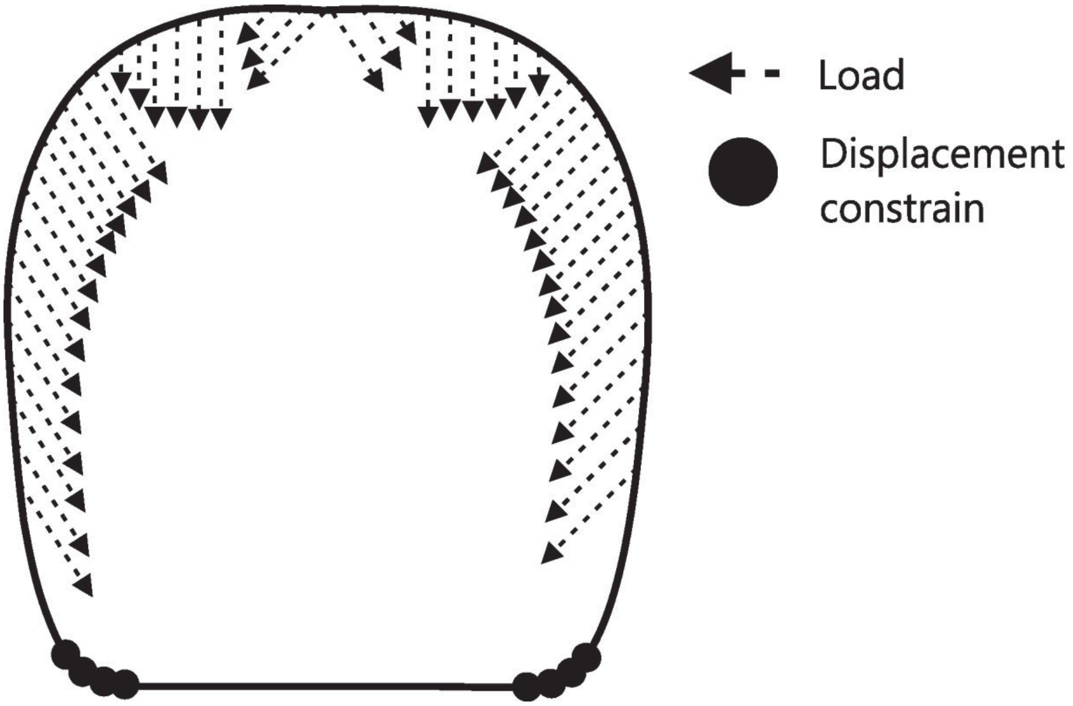

Fig.1. Diagram depicting the boundary conditions of *Pheidole* worker FEA simulations. The same load magnitude was applied to each head side. Nodal displacement constraints were applied on the same number of nodes in each head side and simulation and were positioned to approximate the mandible articulation with the head. Inclined load vectors were defined with an inclination of 45º.

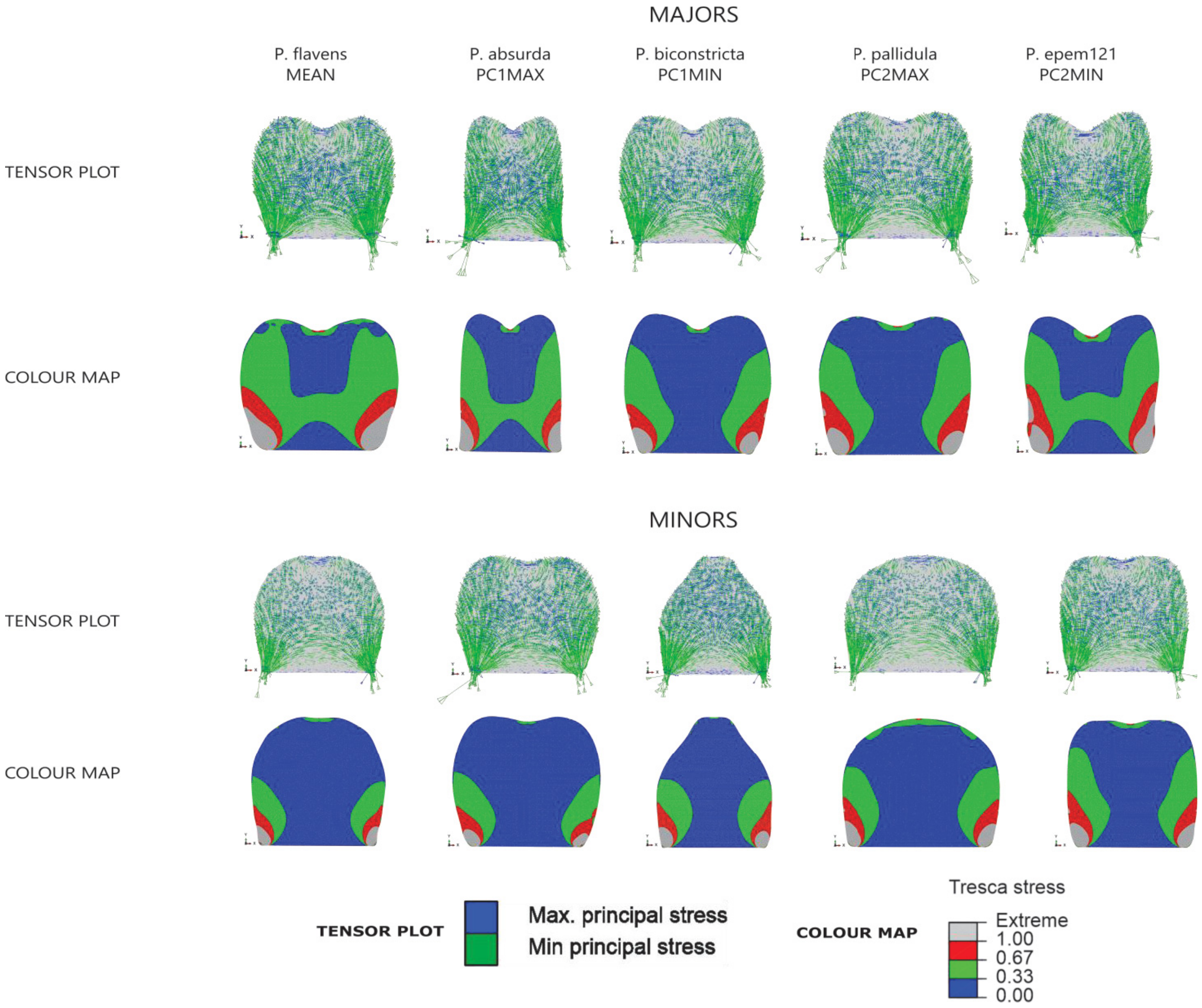

Fig.2. Tensor plots and color maps depicting the results of FEA simulations in *Pheidole* worker 2D heads. The first row of each panel shows tensor plots depicting the distribution of principal stresses. The second row shows the color maps, which depict the variation of proportional stress levels based on the Tresca failure criterion. Tensor plots and color maps have stress limits normalized based on a reference model, and the results for each head shape can be directly compared.

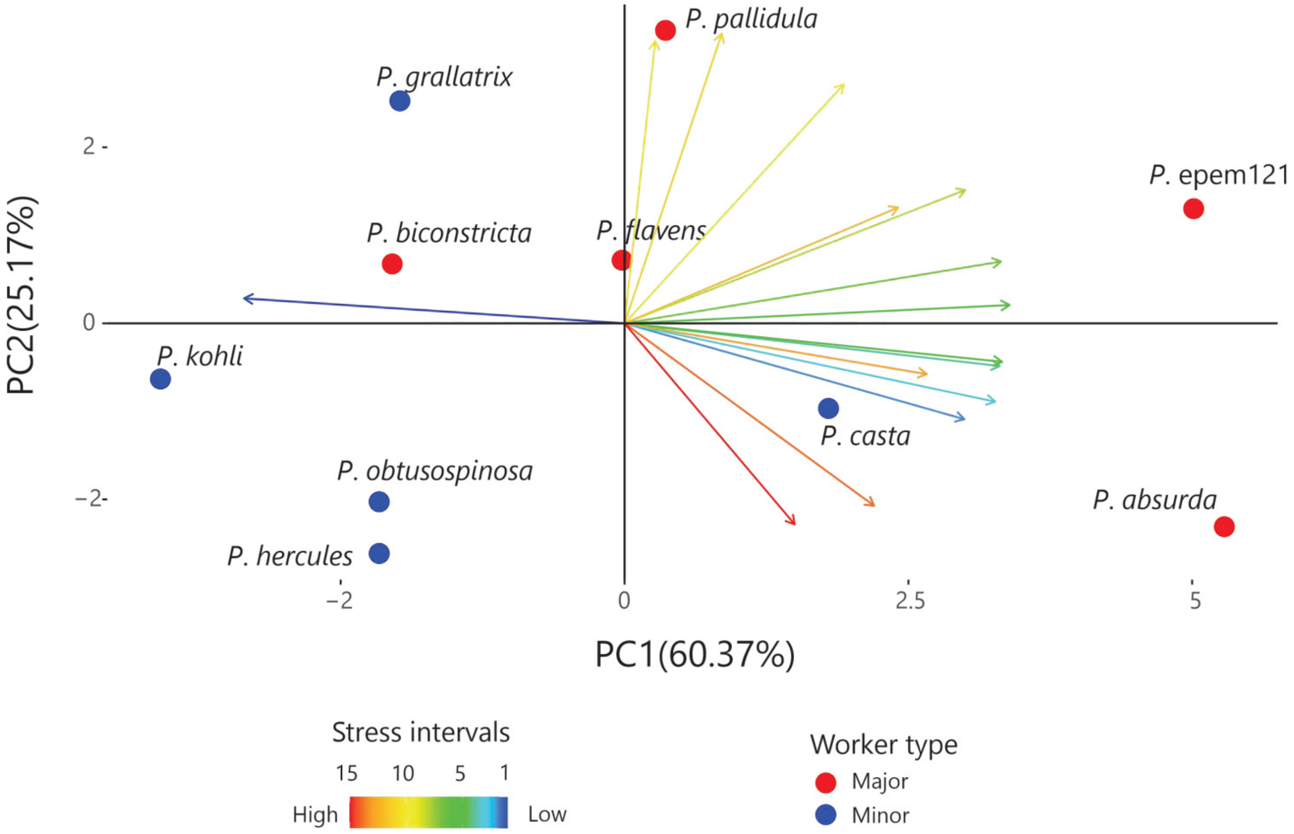

Fig.3. PCA plot showing the differences between *Pheidole* head shapes in the amount of area covered by each of the 15 stress intervals, considering the first two PCA components (see text for details). Stress intervals ranged from 1 (lowest stress levels) to 15 (highest stress levels).

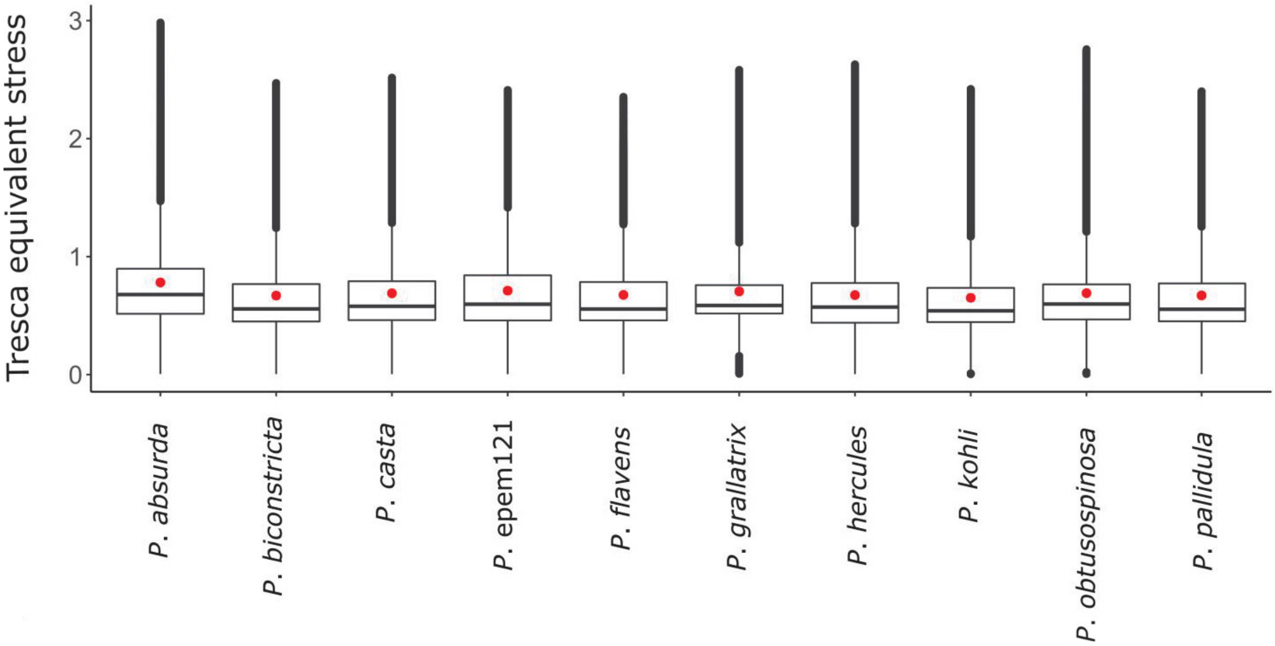

Fig.4. Stress values of each head shape, based on Tresca failure criterion. 2% of higher stress values of each head shape were removed.

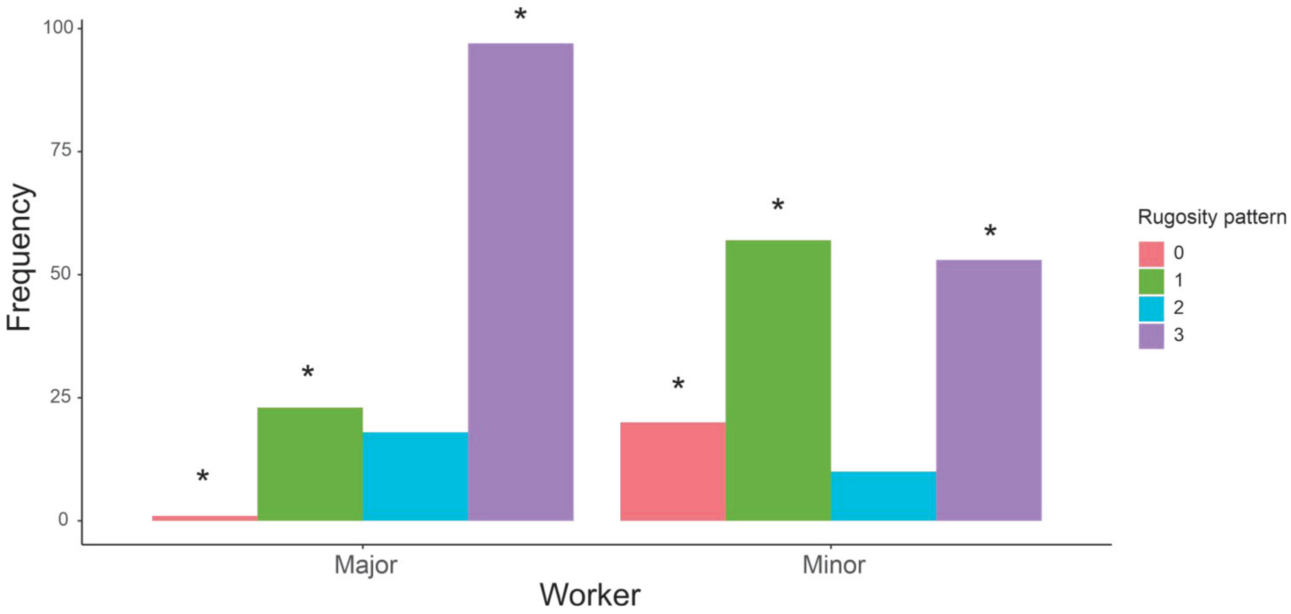

Fig.5. Barplot depicting the frequency of cuticle rugosity patterns in the head of *Pheidole* major and minor workers. (*) denotes that the frequency of rugosity pattern differs between worker type, based on a chi-squared pos-hoc test with Bonferroni correction (Table 5).

4. **MANUSCRIPT 3**

Potential effects of bite loading in ant (Hymenoptera: Formicidae) head shape evolution

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Cristian L. Klunk, Alexandre Casadei-Ferreira and Marcio R. Pie conceived the idea of the manuscript and designed the methodology; Cristian L. Klunk and Alexandre Casadei-Ferreira collected the data; Cristian L. Klunk, Alexandre Casadei-Ferreira, and Marco A. Argenta analyzed the data; Cristian L. Klunk and Marcio R. Pie led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

4.1 Abstract

Coupled with significant lineage diversification, ants also show impressive morphological disparity, notably among workers. Workers rely on mandibles to run most of the colony tasks, which depend on the versatility of the mandible closer muscle (0md1) to be efficient. The 0md1 occupies most of the internal volume of a worker's head, and the head shape can be optimized to improve its volume to harbor larger muscles. Stresses generated by 0md1 contraction potentially impose a risk of cuticular failure on the head capsule, something poorly explored along the diversity of ant head shapes. We quantify ant worker plane head shape diversity and test how it influences the dissipation of stress generated by the 0md1 contraction. Head images of 763 ant species, 318 genera, and 17 subfamilies were collected, and we digitalized four landmarks and 50 semi-landmarks to represent the ant worker's plane head shape. Principal Component Analysis (PCA) was performed to visualize shape variation, and we built a phylogenetic morphospace to investigate its evolutionary trend. We considered extreme and mean shapes of PC1 and PC2 to simulate the 0md1 contraction by Finite Element Analysis and test how head shape affects stress dissipation. The first PCA axis explained 59% of morphological variation and describes the variation in head width. The second PC explained 23% of morphological variation and is related to the depression on the vertexal margin. Regarding the phylomorphospace, PC1 explained 58% of the variance, whereas PC2 explained 26%. Plane heads with deep vertexal depression and closely spaced anteriorly showed more homogeneous stress dissipation patterns, with higher stress levels along the lateral margins of the head. Alternatively, the plane heads with vertexal margins broadly convex and widely spaced anteriorly concentrate more stress on the mandibular insertion, being more susceptible to cuticular failure with increasing bite forces. The PCA mean plane shape showed a compromise in mechanical performance concerning the remaining shapes, avoiding heavy stress concentrations around the mandibular articulations while impairing the spread of high-stress levels along a large area of the head. These results suggest that although spreading stresses throughout a large surface area could be advantageous to avoid cuticular failure, most current ant lineages have a plane head shape that tends to concentrate relevant stress levels around the mandibular insertions, although being able to spread stresses along a wide head area.

4.2 INTRODUCTION

Ants represent a diverse insect lineage, consisting of a hymenopteran family with more than 14,000 species described so far (BOLTON, 2023). Beyond this relevant taxonomical richness, ants also draw attention due to their morphological diversity. Regarding ant external morphology, the diversification of head and mandible shapes along ant lineages suggest potential functional aspects that influence the morphological evolution of these insects. Ant workers rely on their mandibles to execute most colony tasks, which involve behaviors such as biting, excavating, carrying, cutting, and fighting (WHEELER, 1910; WILSON, 1987). Indeed, the versatility of ant mandibles potentially represents one of the main characteristics of their ecological success (WILSON, 1987). Mandibular movement is performed by two muscle pairs located inside the head capsule, but the mandibular adductor muscles (0md1) are much more developed and occupy most of the head's internal volume (PAUL, 2001; PAUL; GRONENBERG, 2002; LILLICO-OUACHOUR et al., 2018; RICHTER et al., 2019; 2020; 2021; BOUDINOT et al., 2021; PÜFFEL et al., 2021).

The head shape seems to be relevant in the context of muscle packing. Broader and heart-shaped heads seem to allow for the storage of more muscle fibers, allowing for an enlarged 0md1 without the need of proportional increases in head size that could be impaired by biomechanical constraints and energetic budgets (PÜFFEL et al. 2021; 2023a; 2023b, PAUL; GRONENBERG, 1999; PAUL, 2001). However, the head capsule provides more than space for muscle storage, offering mechanical support for muscle contraction. Therefore, it is expected that the 0md1 contraction exerts relevant mechanical demands on the ant head capsule, given the degree of the 0md1 development in workers. Since muscle volume and bite force are closely related (BLANKE, 2019; RÜHR et al., 2022), increases in 0md1 volume should be associated with higher mechanical demands in the head capsule.

Besides the awareness of head morphological variation in ant workers and some of its potential functional implications, studies on the evolution and biomechanical relevance of ant head morphology are usually limited to a single lineage (CASADEI-FERREIRA et al., 2021; 2022; KLUNK et al., 2023) or a few model species (PÜFFEL et al., 2021; 2023a; 2023b, PAUL; GRONENBERG, 1999; PAUL, 2001). To investigate the morphological evolution of ant worker heads, it is critical to quantify their diversity with tools such as geometric morphometrics (ZELDITCH et al., 2004). By doing so, it is possible to generate idealized head shapes representing different regions of the head morphospace. Such head shapes could then be employed to explore their functional performance, investigate relevant aspects of head morphology in the context of the mechanical demands associated with the 0md1 contraction, as well as observe potential biomechanical limitations of head shapes that occupy poorly explored regions of the morphospace (KLUNK et al., 2023). This could be achieved by employing Finite Element Analysis (FEA), a numerical method usually applied to simulate the mechanical responses of a structure to distinct loading demands (RAYFIELD, 2007; KUPCZIK, 2008).

Here we accessed ant head morphology evolution and biomechanics by quantifying the head shape variation of most current ant genera through 2D geometric morphometrics, along with biomechanical simulations on plane head shapes that represent different regions of the morphospace. Although overlooking the full complexity of a biological structure, employing 2D data in biomechanical simulations proved effective as a first approximation to the mechanical demands of many complex structures (MARCÉ-NOGUÉ et al., 2013), as demonstrated for the effects of biting behavior in crocodilians (PIERCE et al., 2008; 2009), theropods (RAYFIELD, 2005) and *Tyrannosaurus rex* (RAYFIELD, 2004) skulls, bite loading in vertebrate jaws (DEAKING et al., 2022), the mechanical consequences of borrowing on the trilobite cephalic region (ESTEVE et al., 2021), as well as the effects of 0md1 contraction on *Pheidole* worker plane heads (KLUNK et al., 2023). Given previous studies on the morphological evolution of *Pheidole* worker's head (CASADEI-FERREIRA et al., 2021; 2022), we hypothesize that head shape variation will be substantially related to head width, height, and the degree of vertexal depression. We also expect that ant plane head shapes located at poorly explored regions of the morphospace have biomechanical limitations that prevent their broad spread in current ant lineages. Moreover, we expect the mean head shape to be optimized for bite mechanics, enabling stress to spread along wider head regions instead of promoting stress concentration that could lead to cuticle failure.

4.3 MATERIALS AND METHODS

4.3.1 *Data collection*

We obtained high-resolution images of ant normal workers in full-face view from the online repository antweb.org (ANTWEB, 2021). We downloaded head images of up to three species for each ant genus, whenever those images were adequate to visualize the position of the landmarks and semilandmarks. When images of more than three species were available, we followed the alphabetic order to define the species to represent the genus, since we do not expect relevant variation in general head shape for most current ant genus. We collected images from 783 ant species, representing 318 genera and 16 subfamilies. This sampling effort represents nearly 92% of currently recognized ant genera and around 5.5% of currently recognized ant species (BOLTON 2023).

4.3.2 *Geometric Morphometrics (GM)*

To measure head shape, we digitize four landmarks and 50 semilandmarks (25 on each head side) in the software tpsDig v.2.31 (ROHLF, 2017) (Fig. 1a). Landmarks L1 and L2 represent the confluence of the lateral and posterior margins of the clypeus, L3 represents the midpoint on the clypeus posterior margin, and L4 marks the vertexal margin central point, which consists on a line that divides the head into two equal parts. Semilandmarks were employed to delimit the head contour on both head sides (Fig. 1a). Geometric morphometrics analyses were performed in R version 4.1.0 (R CORE TEAM, 2022) using the geomorph package v. 4.0.4 (BAKEN et al., 2021). We used Procrustes superimposition to remove differences in scale, translation, and rotation. The least-squares criterion (ROHLF; SLICE, 1990) was applied to allow semi-landmarks to slide between fixed landmarks. Left- and right-side landmarks and semi-landmarks were averaged to remove any effect of bilateral symmetry. To correct the variation in head shape by shared evolutionary history, we generate a phylomorphospace based on the ant phylogeny developed by Divieso et al. (2020). Raw data was pruned to drop genera not represented in the phylogeny using the GEIGER package v. 2.0.10 (PENNELL et al., 2014). Principal component analysis (PCA) was used to extract the significant component of variation in head shape, and the phylogeny was projected into the PCA to produce the phylomorphospace (SIDLAUSKAS, 2008). To visualize shape variation, we placed distorted shapes into the background of the phylomorphospace following the code developed by Olsen (2017).

4.3.3 *Finite Element Analysis (FEA)*

We apply FEA to investigate the influence of plane head shape variation in the mechanical demands of the mandibular closing muscle (0md1) contraction. We considered nine idealized plane head shapes spanning the limits of the morphospace, representing the mean and boundary plane shapes of PC1 and PC2, which were generated using the thin-plate spline deformation grids approach (BOOKSTEIN, 1991) in R v. 4.1.0 (R CORE TEAM, 2022). The images of head shapes were vectorized in the software Inkscape, meshed in Fusion 360 (AUTODESK), and than imported into the finite element solver Abaqus 6 (Dassault Systèmes), where sharp edges were smoothed and 2D geometries were defined to proceed with the simulations. We performed linear static simulations with a plane stress approach, which considers that the structure has two main dimensions and the stresses in the third dimension are negligible (MARCÉ-NOGUÉ et al., 2013). Therefore, we apply a unitary and constant thickness to each head shape representation. We designed the finite element mesh of each head shape with plane triangular and quadratic elements (CPS6M), and maintained a similar mesh density for each model (Table 1). We define Young's modulus of the head cuticle as 2.75 GPa, according to measurements taken from leaf-cutting ant mandibles (BRITO et al., 2017), and the Poisson ratio as 0.3, as commonly considered for ant cuticles (LARABEE et al., 2018; ZHANG et al., 2020; KLUNK et al., 2021; WANG et al., 2022). We considered the material properties of the head cuticle as isotropic and linearly elastic.

We simulated the contraction of the 0md1 by applying the load at the nodes of the posterior and lateral margins of each head side, with load orientation approximating the main direction of 0md1 muscular fibers in ant workers (Fig. 1b; MUSCEDERE et al., 2011; LILLICO-OUACHOUR et al., 2018; RICHTER et al., 2019; 2020; 2021; PÜFFEL et al., 2023a). A 1N load was applied in each head side in the model with largest surface area, and the loading magnitude of the remaining models was corrected based on the difference in surface area from that reference model (Table 1; MARCÉ-NOGUÉ et al., 2013). To simulate the bite reaction forces exercised by the mandibles on the head, we fixed as zero the nodal displacement along the x and y directions on head regions that correspond to the points of mandibular articulation, fixing the same number of nodes for each head representation (Fig. 1b).

We considered two plot types to analyze stress patterns: tensor plots and color maps. Tensor plots depict stress distribution through arrows that indicate normalized stress value (arrow size), stress direction (arrow orientation), and stress type (compressive with inward-pointed arrowheads, tensile with outward-pointed arrowheads).

Here we considered minimum and maximum principal stresses in tensor plots, to show stress patterns in the two planes where shear stress is zero. Color maps depict stress patterns by considering a failure criterion, which defines a unique stress value for each mesh element based on the material failure behavior. We chose the Tresca failure criterion to define stress values, which is more conservative and assumes that material failure occurs due to shear stress (ÖZKAYA et al., 2017).

4.3.4 *Intervals method*

We applied the intervals method to evaluate how worker plane head shapes differ in the area covered by distinct ranges of stress values (MARCÉ-NOGUÉ et al., 2017). To perform the intervals method, we extracted element Tresca equivalent stress and area values from Abaqus 6 (Dassault Systèmes) and removed elements of the 2% highest stress values for each simulation since they usually represent artificial high values (MARCÉ-NOGUÉ et al., 2016; 2017). We log-transformed stress values before generating the stress intervals to account for variation between head shapes in the stress scale. The upper threshold was defined as 0.77 so that only 2% of the higher stress values from all simulations were contained in the interval of highest stress (MARCÉ-NOGUÉ et al., 2017). We generated datasets with distinct intervals (5, 10, 15, 25, 50, and 75) and performed a PCA with each dataset to define the ideal number of stress intervals. The scores of PC1 and PC2 of each dataset were used as variables in linear regressions with the scores of equivalent PCs of the next interval (e.g., $PC1_{5intervals} \sim PC1_{15intervals}$), and the coefficient of determination (R^2) was considered to analyze the convergence of PC scores (MARCÉ-NOGUÉ et al., 2017). When R^2 ceased to increase, convergence was achieved and the dataset with the lower number of intervals was chose to proceed with the analysis (MARCÉ-NOGUÉ et al., 2017). Convergence occurred with 15 intervals, therefore we will discuss the results from the PCA with 15 stress intervals. Analyses were carried out in R version 4.1.3 (R CORE TEAM, 2022). We use the R packages FactoMineR version 2.4 (LÊ et al., 2008) and factoextra version 1.0.7.999 (KASSAMBARA; MUNDT, 2020) to perform the PCA.

4.4 RESULTS

4.4.1 *Geometric morphometrics*

The first two axes of the PCA representing the ant plane head morphospace explained 81% of the variance (Fig.2). PC1 explained 59% of the variance and depicts a pattern of decrease in head width and increase in head height (Fig.2). PC2 explained 23% of the variance and is associated with a change in the curvature of the posterior margin of the head, from a concave posterior margin in its negative range towards a convex posterior margin with a deep vertexal depression on its positive range (Fig.2). Most ant plane head shapes are located in the lower left region of the morphospace, being represented by a plane head shape with its width subtly larger than its height, or as width as tall, showing a nearly straight posterior margin of the head (Fig.2). Most ant subfamilies concentrate all specimens around this general plane head shape pattern, exploring a relatively narrow area of the morphospace, as is the case of Pseudomyrmicinae, Myrmiciinae and Ectatomminae. Myrmicinae is the most diverse ant subfamily and accordingly showed a pattern of large morphospace occupation, exploring the ends of the two PCs (Fig.2). No other ant subfamily explores the morphospace beyond the limits imposed by Myrmicinae species, and a substantial part of the morphological diversity of those subfamilies is encompassed into Myrmicinae’s range (Fig.2).

After normalized for the effects of the species phylogenetic relationships, the majority of subfamilies were again densely packed around the general worker plane head shape, except for some lineages that spread towards the positive ends of PC1 and PC2 (Fig.3). Namely, the genus *Daceton* spp. (Myrmicinae) represents a poorly explored plane head shape on the positive range of PC2. *Acanthognathus* spp. (Myrmicinae) explores the end of the PC1 positive range, whereas *Perissomyrmex* spp., *Pristomyrmex* spp., and *Dolopomyrmex* spp. (Myrmicinae) explore the PC1 negative range. Interestingly, however, a Ponerinae (*Odontomachus* spp.) and a Dolichoderinae (*Leptomyrmex* spp.) lineages explored the PC2 negative range beyond Myrmicinae (Fig.3), different from the pattern observed without phylogenetic normalization (Fig.2).

4.4.2 *Finite element analysis*

Our simulations revealed some interesting trends regarding the morphological variability measured by the geometric morphometric approach. As plane heads became wider, stresses concentrate more along their lateral margins and less on their central region (Fig.4). Also, the narrower and wider plane head shapes tended to have higher levels of traction stresses around the vertexal region than averagely wide heads (Fig.4). Those results showed that heart-shaped and narrower heads have higher compressive stresses along the head lateral margins, which suggests a better capacity to spread stresses toward a wider head area than the remaining plane head shapes, which tend to concentrate stresses around the regions of mandibular articulation (Fig.4). Also, narrower and convex heads, but especially the wider heads with deep vertexal depression, need to deal with higher levels of traction stress around their vertexal depression than the remaining head shapes, which could demand some cuticular reinforcement, representing head shapes much less explored in current ant lineages.

4.4.3. *Intervals method*

We performed a PCA with Tresca equivalent stress values to analyze the distribution of non-normalized stress values on plane head models. The first two components explained 86% of the variance (Fig.5) and were considered here for further discussion. PC1 explained 65% of the variance, being deeply associated with a range of stress values from the lowest (1) towards intermediate stress (10) intervals (Fig.5). Its positive spectrum is deeply affected by the stress distribution of the PC1maxPC2max shape, which showed a large head area covered with intermediate stresses. Its negative range is deeply associated with PC1minPC2mean (Fig.5), which has a large portion of the head covered with the lowest stress interval. The second component explained 21% of the variance and is deeply associated with the highest stress intervals (Fig.5). Shapes PC1minPC2min, PC1meanPC2min, and PC1maxPC2mean predominantly influenced the PC2, with the first two located near the end of the positive range, meaning that its surface area is widely covered with the higher stress intervals, while the second was related to a narrower coverage of those intervals (Fig.5).

4.5 DISCUSSION

Our results demonstrated that most current ant lineages show head shapes densely packed around a reduced region of the ant head morphospace, with some lineages, mainly from the subfamily Myrmicinae, exploring wide extensions of the morphospace and approaching its boundaries. Among the current ant lineages exploring the limits of the morphospace, the genus *Daceton* spp. (Myrmicinae) draws attention by being distinctly isolated in the phylomorphospace, having a broad head with a deep vertexal depression. *Odontomachus* spp. (Ponerinae) and *Leptomyrmex* spp. (Dolichoderinae) have taller than wider heads, with the posterior margin of the head narrower than the anterior margin, without a deep vertexal depression. *Acanthognathus* spp. (Myrmicinae) have a narrow and heart-shaped head, whereas the heads of *Perissomyrmex* spp., *Pristomyrmex* spp., and *Dolopomyrmex* spp. (Myrmicinae) are broader and shorter, showing a more convex posterior margin. *Pristomyrmex* spp. and *Perissomyrmex* spp. are closely related genera, while *Dolopomyrmex* spp. represents a distant Myrmicinae clade, suggesting that the resemblance in plane head shape of those genera is a consequence of a common origin (for *Pristomyrmex* spp. and *Perissomyrmex* spp.) and convergence (for *Dolopomyrmex* spp.). On the other hand, *Daceton* spp. and *Acanthognathus* spp. are closely related species whose head shapes differ substantially, suggesting the potential for dramatic divergencies in ant worker head shape in short evolutionary timescales.

Results from the tensor plots and intervals method suggest a relevant variation in the mechanical behavior of ant worker head shapes along the morphospace. Head shapes PC1maxPC2max and PC1minPC2min, which lie on opposite sides of both morphospace axes, showed a large portion of their surface area covered with intermediate to high non-normalized stress levels. However, the stress patterns depicted by such head shapes are sharply contrasting. While PC1maxPC2max showed proportionally higher stress levels spreading towards most of its surface area, especially along the lateral margins with a high stress concentration on the vertexal depression, in PC1minPC2min the highest stress levels concentrate around the regions of mandibular articulation, with the central and lateral regions of the head showing proportionally lower stresses than in PC1maxPC2max. Both shapes lie on unexplored portions of the phylomorphospace, suggesting that higher stresses, covering or not most of the head surface area, represent possible mechanical limitations. The shape PC1minPC2mean, otherwise, showed most of its surface area covered with the lowest stress intervals, along with a stress pattern where proportionally higher stresses concentrate around the mandibular articulations, which could help to explain why such head shape rarely occurs in current ant species. Interestingly, the most explored head shape among modern lineages, the mean shape, have its head area covered predominantly by low levels of stress, although allowing a more wide spread of high-stress levels.

Ant workers rely on their mandibles to perform the colony tasks. Therefore, ecological aspects such as prey capture, food processing, defense, and nest construction represent potential pressures on ant head shape evolution. Considering the genus *Pheidole*, there is no evidence that major workers of granivore species have larger heads than non-seed-eating species, but the intraspecific difference in head size between minor and major workers was higher in species that eat seeds (HOLLEY et al., 2016). General feeding and nesting habits of *Pheidole* species were not correlated with worker head shape when evaluated under a geometric morphometric approach (CASADEI-FERREIRA et al., 2021) but seem to be related to the geographic distribution of the genus, with species within the east and west hemispheres converging in head shape (CASADEI-FERREIRA et al., 2022). Although worker head shape seems to evolve more independently between worker subcastes than the mesosoma (FRIEDMAN et al., 2019; 2020), which could be a response to the different functional roles of each worker type in colony maintenance, the evidence so far suggests that head shape evolution in *Pheidole* is driven mainly by intrinsic factors, such as the variation in size (PIE; TRANIELLO, 2007; PIE; TSCHÁ, 2013; CASADEI-FERREIRA et al., 2022). In *Solenopsis geminata* and *Pogonomyrmex badius*, there is evidence that granivory droves worker polymorphism in head morphology (FERSTER et al., 2006). In *Cephalotes* spp., a worker subcaste is specialized in protecting nest entrances with its unusually enlarged and distinctly shaped head (POWELL, 2008; 2009). In the army ants of the genus *Eciton* it was suggested that the evolution of a sub-major worker type with an enlarged head is associated with the increase in the diversity of prey types since such workers are responsible for prey transport (POWELL; FRANKS, 2006). Recently it was hypothesized that males of the genus *Dorylus* spp. have head shapes tuned to provide more space to the 0md1 and improve the mechanical advantage of the mandibular lever system, a result of sexual selection associated with the necessity to grip the female neck during sexual intercourse (BOUDINOT et al., 2021). In some morphologically disparate ant fossil lineages, head morphology seemed to be relevant to successfully capturing and processing prey (BARDEN et al., 2020). Trap-jaw mechanisms evolved several times in *Strumigenys* spp., with a profound effect on species feeding habits and usually associated with morphological modifications on workers' heads, mainly around the mandibular articulations (BOOHER et al., 2021). In a broader taxonomic coverage, head measurements showed weak relevance in predicting ant ecological and functional characteristics (SOSIAK; BARDEN, 2021), even when adding fossil lineages (SOSIAK et al., 2022).

All those studies provide mixed conclusions about the importance of head shape for ant species ecology. Although valuable approaches, most lack an explicit biomechanical focus to link head shape with function. Since the mandibular muscles originate inside the head capsule in insects, head size and bite force are usually highly correlated due to the 0md1 volume increase with head size (BLANKE 2019; RÜHR ET AL., 2022). Most previous assessments of the functional aspects regarding head morphological variation in ants highlighted the importance of head shape modification to an increase in 0md1 volume (GRONENBERG et al., 1997; PAUL; GRONENBERG, 1999; PAUL, 2001; LILLICO-OUACHOUR et al., 2018; PÜFFEL et al., 2021; 2023a; 2023b). Head width and height increase represent relevant morphological modifications to improve bite force capacity in ant workers (PÜFFEL et al., 2021; 2023a). Broader (GRONENBERG et al., 1997; PAUL; GRONENBERG 1999; PAUL, 2001) and especially heart-shaped heads (PÜFFEL et al., 2021; 2023a) represent morphologies better suited to accommodate larger muscles that generate powerful bites, whereas longer and slender heads hypothetically benefit faster muscular contractions (GRONENBERG et al. 1997; PAUL; GRONENBERG, 1999; PAUL, 2001). Besides the influence of the endoskeleton and cuticular ridges (BLANKE et al., 2017a; 2018), the head capsule shape also influences stress dissipation in insects, as demonstrated for *Pheidole* ants (KLUNK et al., 2023). Similarly to the observed here, heart-shaped heads of *Pheidole* major workers are more prone to spread stresses toward the head than the general head shapes of minors, which could be advantageous to deal with more powerful bites because it avoids heavy stress concentrations (KLUNK et al., 2023).

By applying a quantitative morphological approach, coupled with biomechanical simulations, we were able to quantify the morphological diversity in ant worker head shape and unveil general mechanical patterns associated with the main plane shapes represented in the morphospace. Most of the poorly explored idealized plane head shapes show signs of biomechanical limitations, like a heavy concentration of stress in a reduced head area, or the spread of the higher stress intervals in a broader head area. In contrast, the most currently explored plane head shape, the mean shape, shows a compromise between concentration and spread of the higher stress levels. A better understanding of ant head shape evolution and the functional determinants of head morphological variation calls for studies considering the effects of 3D head shape, as well as other aspects of head morphology that are relevant in biting context, such as the roles of the endoskeleton and cuticular reinforcements on stress dissipation (BLANKE et al., 2017a; 2018).

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4.7 Tables and figures

Table 1. Position in the morphospace, mesh area, number of elements and load applied on each head side of the models chose for FEA.

Morphospace position	**Mesh area (mm^2)**	**Number of elements**	**Load (N)**
PC1maxPC2max	2.16	73119	0.84
PC1maxPC2mean	3.03	73578	1.00
PC1maxPC2min	2.72	72536	0.95
PC1meanPC2max	1.83	71785	0.78
PC1meanPC2mean	2.43	73708	0.90
PC1meanPC2min	2.43	71781	0.90
PC1minPC2max	1.44	71990	0.70
PC1minPC2mean	1.85	71110	0.78
PC1minPC2min	1.52	71986	0.71

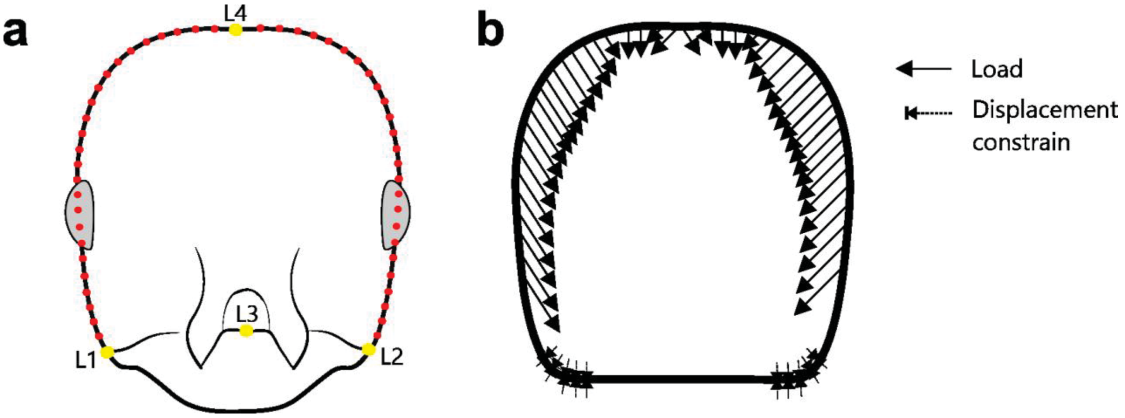

Fig.1. Diagram depicting landmarks (yellow dots) and semilandmarks (red dots) distribution to quantify the morphological variation in ant worker heads (a); disposition of loaded and fixed regions (displacement constrains) on the worker head for FEA (b).

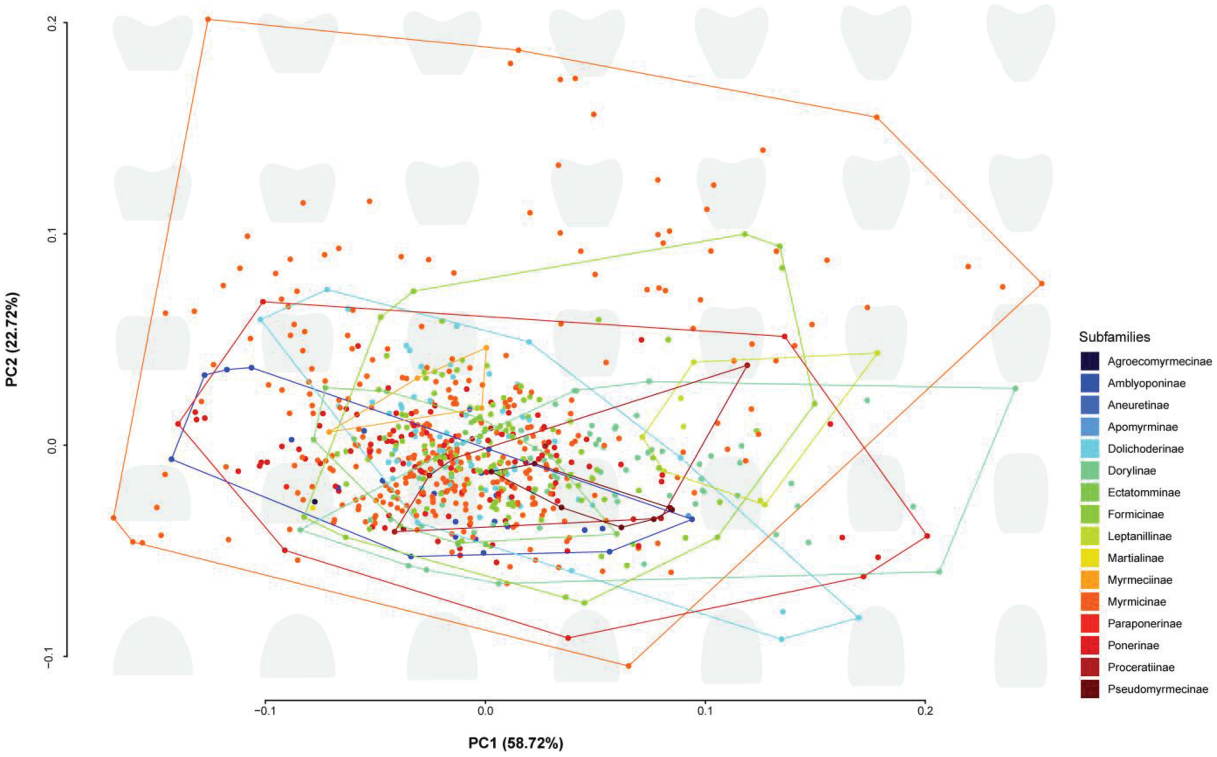

Fig.2. PCA representing the morphospace of ant worker 2D head shapes, without allometric effect.

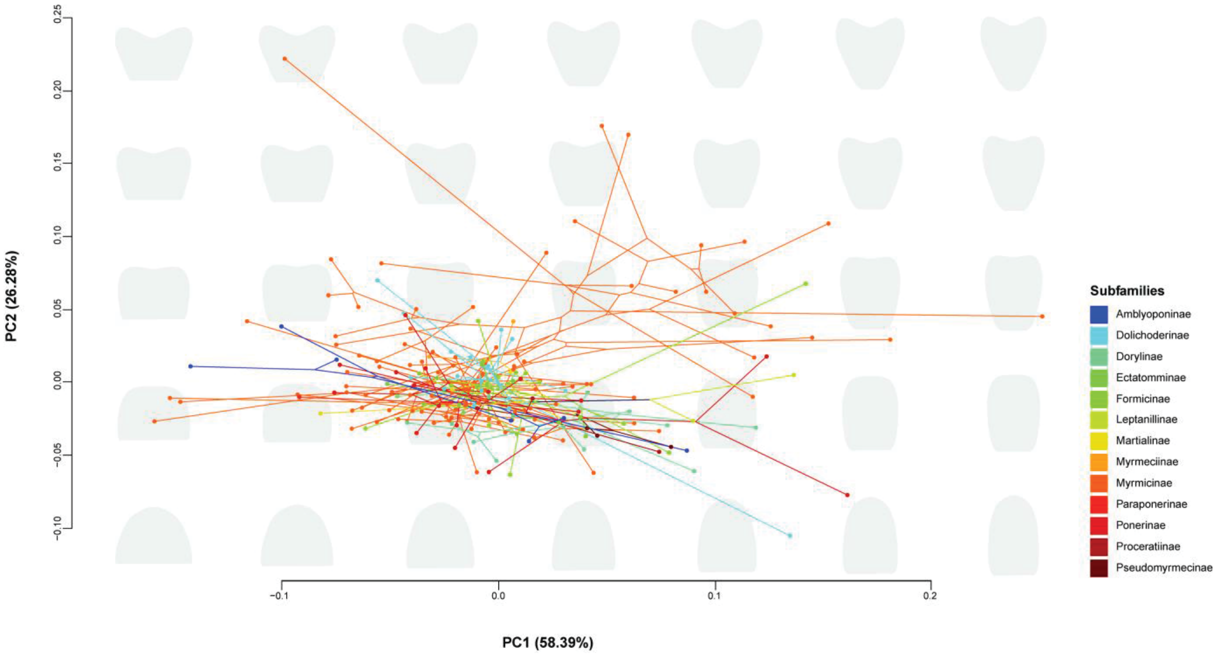

Fig.3. Phylomorphospace of ant worker 2D head shapes, without the effect of allometry.

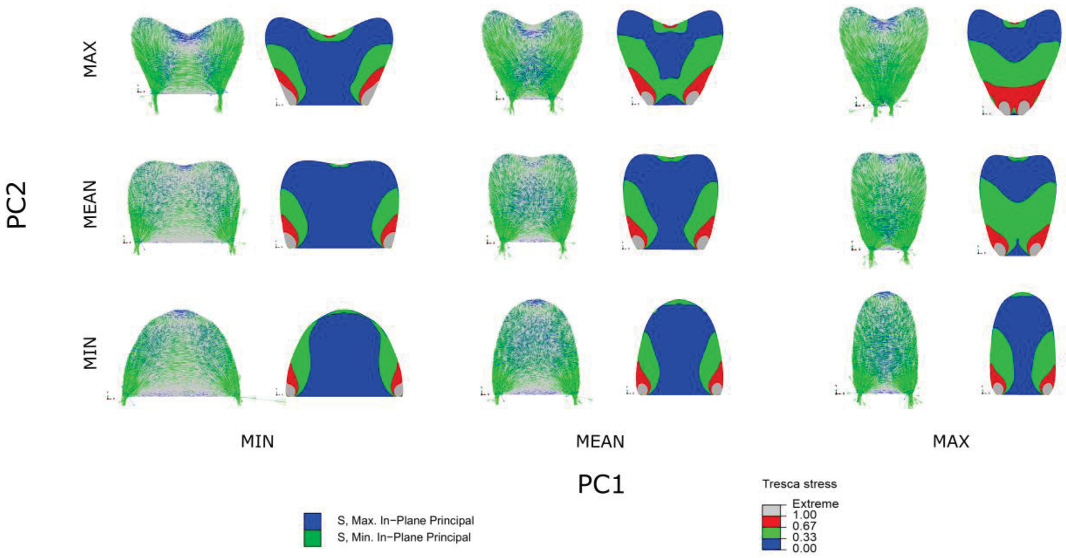

Fig.4. Tensor plots and colour maps depicting normalized stress distribution on idealized ant worker head shapes. Tensor plots depict normalized stress magnitude, direction and type (compression or traction) of maximum and minimum stresses, which represent stresses on specific planes were shear stress is zero. Color maps depict normalized Tresca equivalent stress values, without information of stress direction and orientation.

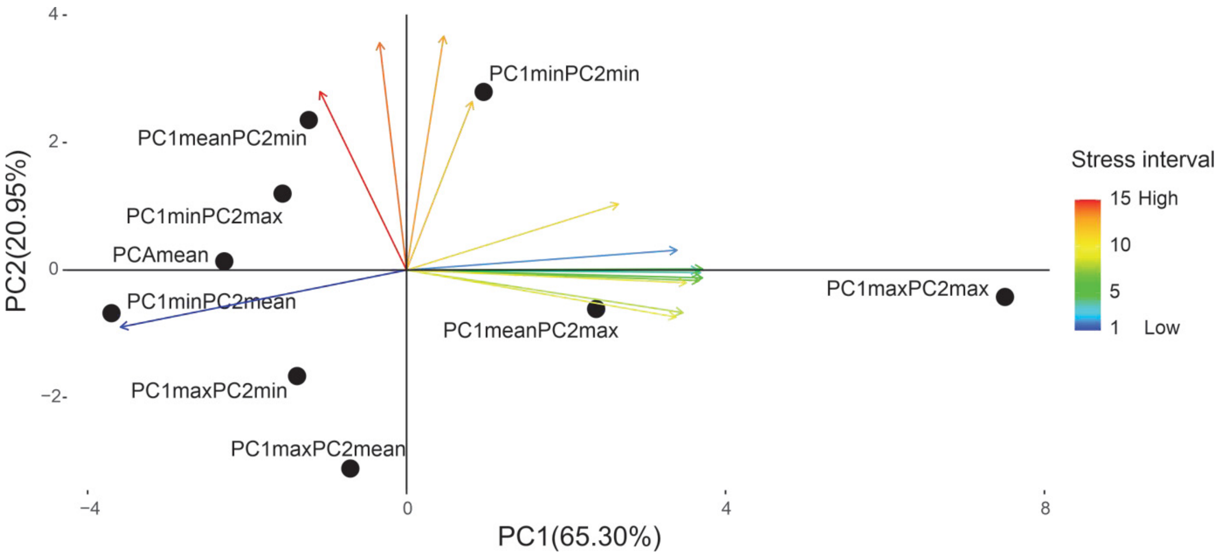

Fig.5. PCAs depicting the distribution of head shapes considering the amount of surface area covered with each of 15 stress intervals, which represent non-normalized and log transformed stress values based on the Tresca failure criteria.

5. **MANUSCRIPT 4**

Simulated biomechanical performance of morphologically disparate ant mandibles under bite loading

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Cristian L. Klunk and Marcio R. Pie conceived the idea of the manuscript. Cristian L. Klunk, Marcio R. Pie, and Marco A. Argenta designed the methodology; Félix Rosumek, Thomas Van de Kamp and Jörg Hammel collected the data; Cristian L. Klunk and Marco A. Argenta analyzed the data; Cristian L. Klunk and Michael Heethoff led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

5.1 Abstract

Food processing can exert significant pressure on the morphological evolution of animal mouthparts. Insects evolved various modifications to their ancestral Bauplan of mouthparts, which allowed for a broad exploration of feeding modes. In ant colonies, workers perform non-reproductive tasks, relying heavily on their mandibles. Given the importance of biting for various activities performed by ant workers and the significant morphological diversity of mandibles across different species, it is essential to understand how mandible shape influences the mechanical responses of mandibles to bite loading demands. In this study, we employed Finite Element Analysis to simulate four biting scenarios on volumetric models of worker mandibles from 25 ant species, including omnivorous, generalist, and specialist predator species. We selected species with a broad morphological diversity, which allowed us to explore several lineages within the family. We hypothesize that mandibles of predatory ants would perform better than mandibles of omnivorous species due to their necessity to capture and subdue prey. We normalized the load to control for mandible size and defined the same cuticle material properties for all species, allowing only the mandible morphology to vary between species. Our results demonstrated interspecific differences in the mechanical responses of mandibles to bite loading demands. Some species concentrate stresses in specific mandibular regions, mainly around the articulations, whereas in others, stresses spread along a wider surface area. Most species showed important distinctions in performance between biting scenarios. However, we found no evident differences in stress patterns between predatory and omnivorous ants. These results indicate that ant mandibles are versatile tools used in different contexts in every species and that interspecific variation in mandible morphology has important effects on bite mechanics.

5.2 INTRODUCTION

One crucial aspect of insect feeding is the ability to capture prey and chew food items using mandibles (BLANKE, 2019), which leads to considerable mandibular morphological divergence across species (LABANDEIRA, 2019), and whose relevance is highlighted by the degree of development of mandible adductor muscles (CHAPMAN, 1998). Ant workers must perform various tasks by modulating the force and velocity of their bites, along with employing other mouthparts to further process food, for example. While most ant mandibles are shovel-shaped with a narrow base and a triangular blade (RICHTER et al., 2022), there is considerable interspecific variation in mandibular morphology (WHEELER, 1910; WILSON, 1987), which may reflect functional adaptations to specific feeding habits or other ecological roles (SOSIAK; BARDEN, 2021).

Although some studies have failed to find a clear relationship between mandible shape and function (CASADEI-FERREIRA et al., 2021), there is evidence that particular ant species evolved specialized mandible shapes for specific tasks. For example, *Melissotarsus* ants have mandibles specialized for excavation (KHALIFE et al., 2018), while *Pheidole* spp. major workers have mandibles with a notable ability to exert pressure during a bite (KLUNK et al., 2021). In leaf-cutting ants, the mandible shape of some worker types excels at cutting leaves (CAMARGO et al., 2015; 2016). Sub-major workers of *Eciton* spp. have mandibles that allow for a more powerful grip to carry large prey (POWELL; FRANKS, 2005; 2006). The falcate mandibles of *Dorylus* spp. males seem associated with a better capacity to grip the queen during copulation (BOUDINOT et al., 2021). Ants with power amplification mechanisms, such as trap and snap-jaw ants, have particularly distinctive mandibles that are longer and narrower than remaining ants. Among many functional aspects, this may be an adaptation to withstand the mechanical demands of their powerful strikes (LARABEE et al., 2018; WANG et al., 2022) and facilitate prey capture (OHKAWARA et al., 2016; SOSIAK; BARDEN, 2021). Additionally, the fossil record suggests that disparate mandible shapes were prevalent from the beginning of ant diversification (BARDEN et al., 2020), and shovel-shaped mandible may not be the ancestral condition in ants (BOUDINOT et al., 2022; RICHTER et al., 2022). Some authors argue that the ancestral ant may have relied on a falcate-shaped mandible (BOUDINOT et al., 2021). Overall, understanding the functional implications of mandibular morphological variation in ants is a crucial area of research that can shed light on the ecology and evolution of these insects. In addition to interspecific variation, mandible shape can vary intraspecifically between worker types in polymorphic ant species. There is evidence that variation in the mandible morphology of polymorphic workers is associated with task activity in some ant species (CAMARGO et al., 2015; 2016; LARABEE et al., 2018; KLUNK et al., 2021).

Biomechanical simulations are valuable tools to explore the relationship between mandible morphology and biting performance (HÖRNSCHEMEYER et al., 2013; BLANKE et al., 2017b; GOYENS et al., 2014; 2015; 2016; LARABEE et al., 2018; KLUNK et al., 2021). In recent years, there has been a significant increase in the availability of high-quality morphological data for ants due to improved access to microtomography facilities (high-resolution desktop and synchrotron µCT) (BUSER et al., 2020). It has allowed for a broad application of biomechanical simulations and studies of functional morphology, and has been particularly useful in investigating the relationship between mandible morphology and bite performance. However, in the case of ants, most studies have focused on a few species (LARABEE et al., 2018; ZHANG et al., 2020; KLUNK et al., 2021; WANG et al., 2022).

In this context, our study aimed to investigate the role of mandible shape in bite performance across a wide range of ant species. By biting performance we considered the distribution of stress in solid models of ant mandibles under loading conditions that characterize distinct biting behaviors. Stress is the force that concentrates in a specific area (HIBBELER, 2017). It is a mechanical response of the structure to external loading demands and is a valuable property to consider when comparing the responses of different organisms to similar external loading conditions (RAYFIELD, 2007; KUPCZIK, 2008). We selected ants with different feeding habits, including generalist and specialist predators (trap-jaw ants), omnivorous ants, and one leaf-cutting species. The main focus of our study was to discuss how the variation in mandible morphology influences stress distribution and investigate whether predatory ants, which need to capture and subdue living prey, possess mandibles morphologically specialized in dealing with bite loading. To achieve this, we used Finite Element Analysis (FEA) to simulate biting behaviors in 3D mandible models of several ant species.

Our main hypothesis was that predatory species would perform better (i.e. show proportionally lower levels of stress) than non-predatory species in biting simulations. We also expected that trap-jaw ants would perform better at strike biting than all other species due to their characteristic fast bites. Additionally, we predicted that sturdier mandibles, such as those of major workers in polymorphic species like *Pheidole* spp., would perform better than slender mandibles in simulations of pressure biting (KLUNK et al., 2021), regardless of the main feeding habit of the species. By conducting this comparative study across a wide range of ant species, we aimed to gain a better understanding of the relationship between mandible morphology and bite performance and shed light on the functional implications of mandible variation in ants.

5.3 MATERIAL AND METHODS

5.3.1 *Species selection*

To simulate biting behavior, we selected ant species with a wide range of mandible morphologies, which were representative of two primary feeding habits: predatory and omnivorous (Table 1). To establish the general feeding habits of these species, we collected information from online repositories (e.g., ANTWIKI, 2022) and literature sources (ROSUMEK, 2017; HOUADRIA; MENZEL, 2020). In cases where we did not find the species' main feeding habit or were able to identify the species only at the genus level (morphospecies), we considered the main feeding habit of the genus. We classify as predators the ant species known to actively hunt other animals (mainly arthropods) because of the mechanical demands of subduing a prey, which is assumed to differ from simply feeding on dead animals. For the predatory category, we included species with specialized mandible morphology and mechanics, the trap-jaw ants. Due to their specialized mandibles, these species were classified here as specialized predators, although not necessarily feeding exclusively on live prey. Omnivorous species do not actively predate other organisms as their primary feeding habit, usually relying on many different food sources without subduing living prey. We also added a leaf-cutting ant (*Acromyrmex aspersus*), which exhibits a unique biting behavior for cutting leaves. We aimed to broadly categorize species based on their primary differences in feeding mechanical demand, not describe in detail their natural diets, since many species are known to opportunistically feed on a wide range of trophic sources (ROSUMEK, 2017). In cases where different species displayed similar mandible morphology, we relied on the quality of the µCT scans (see below) to decide which species would enter our final dataset.

5.3.2 *Scanning and reconstruction of volumetric models*

Ant specimens were scanned using synchrotron radiation X-ray tomography at two German facilities, DESY (Hamburg) and KIT Light Source (Eggenstein-Leopoldshafen). The resulting scans were assembled as Tiff image series for subsequent reconstruction, with scanning parameters varying depending on the ant species (Table 1). Volumetric CT data of ant worker mandibles were then pre-segmented using the software Amira 5.4 (Visage Imaging GmbH, Berlin, Germany). A set of slices was manually segmented using the Magic Wand tool, at intervals ranging from five to 15 slices, depending on the complexity of the mandible and the quality of the scans. Automatic interpolation between the pre-segmented slices was done using the online platform Biomedisa (LÖSEL et al., 2020). To ensure that the digital representations adequately represented the morphology of ant mandibles, the outputs from Biomedisa were imported back into Amira 5.4, where we corrected inaccuracies and reduced the complexity of the reconstructed morphology, taking into account the presence of hairs, sharp edges, and holes, among other factors.

5.3.3 *Finite element mesh generation*

The mandible surface models were imported into Blender 2.93 (https://www.blender.org/) to position them in a common orientation. We then used FUSION 360 (AUTODESK) to decrease mesh density. By conducting mesh convergence simulations, we determined a minimum mesh density for each mandible that approximates the 3D mandible morphology and provides a lower computational demand to solve the finite element equations (MARCÉ-NOGUÉ et al., 2015). To emulate actual simulation parameters, we generated a simplified version of the final simulation setup, sampling three to six elements from specific mandibular regions to check for convergence (less than 5% of error) on von Mises stress values (ÖZKAYA et al., 2017). We exported meshes from FUSION 360 as .stl files and imported them into FEBio (MAAS et al., 2012), where we conducted convergence tests and the final FEA simulations. The number of elements for the definitive meshes is available in Table 1.

5.3.4 *Finite Element Analysis*

In this study, we aimed to investigate the effect of mandible morphology on bite-loading demands by simulating four bite conditions that reflect various phases of biting and the employment of different parts of the masticatory margin. Specifically, we simulated mandible use in strike and pressure biting using the entire masticatory margin and employing only the apical tooth. Strike biting emulates the usage of mandibles to hit an object and represents the first phase of bite. To simulate strike biting, we applied a load on the masticatory margin or the apical tooth of the mandible, restricting the nodal displacement on the mandibular articulations with the head in all directions (Fig.1). Similarly, for the second bite phase, which we refer to as pressure biting and which involves crushing or gripping an object, we applied a load on the mandibular region where the mandibular apodeme inserts, following the direction of forces generated by 0md1 contraction. We also restricted the mandible articulations with the head and the masticatory margin or apical tooth to zero nodal displacements in all directions. To ensure consistency, we applied the same material properties to each simulation. The Young's modulus was defined as 2.75 GPa based on measurements from the mandibles of *Atta laevigata* available in the literature (BRITO et al., 2017), and the Poisson ratio was set at 0.3, as commonly considered for ant cuticle (LARABEE et al., 2018; ZHANG et al., 2020; KLUNK et al., 2021; WANG et al., 2022). To account for differences in mandible size, we applied a 1 N load to the largest mandible and adjusted the load for the remaining mandibles based on surface area differences (Table 1) (DUMONT et al., 2009). Restricting parameter variation to mandible morphology alone, such as mandible shape and cuticle thickness, allows us to compare results between simulations (RAYFIELD, 2007). This approach is commonly used in comparative studies (GOYENS et al., 2014; 2015; 2016; BLANKE et al., 2017b; LARABEE et al., 2018; KLUNK et al., 2021).

5.3.5 *Intervals Method*

We applied the intervals method independently to each biting scenario. We extracted data on von Mises stress and volume from the elements of each simulation from FEBio (MAAS et al., 2012). Then we removed elements that corresponded to the 2% higher stress values in each simulation, as these values often represent artificially high-stress values (MARCÉ-NOGUÉ et al., 2016; 2017). To account for mandible variation in the stress scale, we log-transformed stress values prior to generate stress intervals. We defined the upper threshold values for each biting scenario to include the 15% highest stress values above the threshold. Defining a higher threshold value could lead to uninformative intervals, as many models would have no elements within the highest interval due to the variation in the range of stress generated among models. To define the ideal number of stress intervals, we generated datasets with different numbers of intervals (5, 10, 15, 25, 50) and performed PCAs. We considered PC1 and PC2 scores of each dataset in linear regressions with the scores of equivalent PCs of the next interval (e.g., $PC1_{5\_intervals} \sim PC1_{10\_intervals}$), and we retrieved the coefficient of determination (R^2) to analyze the convergence of PC scores. The stop of increase in R^2 defined the final number of intervals (MARCÉ-NOGUÉ et al., 2017). For all biting scenarios, convergence occurred with 15 intervals, so we used this number for the PCAs. We conducted our analyses in R version 4.1.3 (R CORE TEAM, 2022) using the FactoMineR version 2.4 (LÊ et al., 2008) and factoextra version 1.0.7.999 (KASSAMBARA; MUNDT, 2020) packages.

To summarize, we provide our FEA results in two ways: colour maps and the intervals method (MARCÉ-NOGUÉ et al., 2017). Colour maps were graphical outputs generated by FEA software displaying stress distribution along the mandibles. To compare results between species and biting scenario stress values were normalized using a reference model. The resulting colour variation on the maps represents the proportion of stress based on the maximum normalized stress value for each simulation (Fig.2). In contrast, the intervals method involved quantifying the volume of the mandible that falls within specific stress intervals for each of the four biting scenarios (MARCÉ-NOGUÉ et al., 2017). These proportions served as input variables for the PCAs used to address differences in stress distribution among ant mandibles across the 15 stress intervals (Fig.3).

5.4 RESULTS

5.4.1 *Color maps*

In strike simulations, most mandibles of predatory and omnivorous species concentrate relatively higher stresses around the mandibular articulations with the head, while the stresses were comparatively lower along the mandible blade. Some species, however, showed a large surface area with intermediate to high stress levels along the mandible blade. Among predatory species, *A*. *brevicornis*, the major worker of *Carebara* sp.01, *Lophomyrmex* sp.01, *P*. *cribrinodis*, and *Parasyscia* sp. had high-stress concentrations around the mandibular articulations, whereas *B*. *fugax*, the minor worker of *Carebara* sp.01, the mean worker of *E*. *burchelliii*, *E*. *edentatum*, *M*. *ruginodis*, and *O*.

petiolata also showed high levels of stress along the mandible blade. Similarly, omnivorous species such as *C*. *pusillus*, *F*. *fusca*, *H*. *dentinodis*, *L*. *niger*, *O*. *chelifer*, the major worker of *P*. *aper*, and *W*. *affinis* concentrated high levels of stress around the mandible articulations with the head, while *Azteca* sp., *C*. *zenon*, *Dorymyrmex* sp., the minor worker of *P*. *aper* and *Solenopsis* sp.04 exhibited relatively high stresses along the mandible blade too. Finally, the leaf-cutting species *A*. *aspersus* showed intermediate to high stress levels along the mandible blade (Fig.2a and c).

In terms of pressure biting, several predator species, including the major worker of *Carebara* sp.01, *E*. *edentatum*, *Lophomyrmex* sp.01, *O*. *petiolata*, and *Parasyscia* sp., showed proportionally lower levels of stress along the mandible blade, a pattern found only in the major worker of *P*. *aper* among omnivorous species (Fig.2b and d). Meanwhile, some omnivorous species, such as *Solenopsis* sp.04 and the minor worker of *P*. *aper* showed a large surface area in their mandible blade with proportionally high stress levels (Fig.2b and d). The leaf-cutting ant *A*. *aspersus* was among the species with lower relative stress levels along the mandible blade. Although most species showed relatively higher stress levels covering a larger surface area in pressure than strike biting, there were some important exceptions to this pattern, such as the mandibles of *A*. *aspersus*, *E*. *edentatum*, *O*. *petiolata*, *H*. *striatula*, and the major worker of *Eciton burchellii*, which exhibited only subtle differences in stress distribution between biting conditions (Fig.2).

For simulations using only the apical tooth, we excluded the mandibles of *A*. *brevicornis*, *E*. *burchellii*, *O*. *chelifer*, and *S*. *denticulata* due to their specialized morphologies, in which the independent use of the apical tooth is improbable. In general, when comparing the use of the entire masticatory margin to employing only the apical tooth in strike and pressure simulations, we found no relevant differences (Fig.2). However, *H*. *striatula* and *L*. *foveolatus* represented two exceptions, where using the apical tooth only resulted in a more spread-out distribution of higher stress levels, representing a worse stress pattern (Fig.2c and d).

Ectatomma edentatum and *H*. *striatula*, which are closely related and exhibit similarities in their mandible morphologies, showed clear distinctions in stress distribution in strike and pressure biting. Specifically, *H*. *striatula* showed a larger surface area of the mandible with relatively higher stresses than *E*. *edentatum* (Fig.2). This finding suggests that *E*. *edentatum* exhibits superior biting performance compared to *H*. *striatula*.

5.4.2 *Intervals Method*

To further explore the interspecific differences in mandibular stress distribution, we conducted PCAs for each biting scenario using the percentage of mandibular volume covered by 15 stress intervals as input variables. In strike simulations with the entire masticatory margin, the first two components of the PCA explained 84.57% of the variance. PC1, which accounted for 54.17% of the variance, represented stress intervals ranging from low to high, except for the highest stress interval, which was more closely associated with PC2. An increase in PC1 indicated essentially a larger mandibular volume covered by intervals of low stress (Fig.3a). The negative range of PC1 included *E. burchellii* workers, *O. chelifer*, *S. denticulata* and *A. brevicornis*, all of which had a larger mandibular volume filled with higher stress intervals. In contrast, the positive range of PC1 featured species such as *C. pusillus* and the major workers of *Carebara* sp.01 and *P. aper*, which had a larger mandibular volume filled with the lowest stress intervals (Fig.3a). PC2 explained 30.40% of the variance and mainly distinguished mandibles with a higher coverage of the highest stress levels (interval 15) from the remaining species in its negative range. This pattern was observed in the major worker of *E. burchellii*, *S. denticulata*, and *A. brevicornis*. The positive range of PC2 was associated with an increase of intervals 5-9, but no species or group of species was distinctly isolated in this region of the PCA (Fig.3a).

For simulations of pressure with the entire masticatory margin, the first two components of the PCA accounted for 86.67% of the variance. PC1 explained 45.76% of the variance and primarily distinguished species with a proportionally larger mandibular volume displaying higher stress (such as the major worker of *E. burchellii* and *S. denticulata*) from species more associated with intervals 2-5 (such as *H. striatula*) (Fig.3b). PC2 explained 40.91% of the variance and was positively associated with intervals 8-12. It mainly differentiated *O. petiolata* and *A. brevicornis* from other species in its negative range, as these species had lower proportions of their mandibles filled with such intervals (Fig.3b).

In strike simulations using only the apical tooth, PC1 explained 67.77% of the variance and showed a positive association with intervals 2-7 while being negatively associated with intervals 13-14. Along the negative range of PC1, *L. foveolatus* and the median worker of *E. burchellii* displayed a larger mandibular volume with higher stress levels. In contrast, on the PC1 positive range, *C. pusillus*, the major worker of *Carebara* sp.01 and *P. aper* stood out for having a larger mandibular volume with the lowest stress interval (Fig.3c). PC2 explained 21.60% of the variance and was associated with a larger mandibular volume filled with intervals 9-11. This axis mainly differentiated the major worker of *P. aper* and the median worker of *E. burchellii* from other species in its negative range, given their lower proportion of mandibular volume filled with such stress intervals (Fig.3c).

In simulations of pressure using only the apical tooth, the first two components accounted for 87.22% of the variance. PC1 explained 51.66% of the variance and showed a positive association with intervals 2-4 while being negatively associated with intervals 12-14. This axis mainly differentiated *Azteca* sp., *C. pusillus*, *Parasyscia* sp., and *E. edentatum* from other species in its positive range (Fig.15d). PC2 explained 35.56% of the variance and was positively associated with intervals 7-10. This axis mainly isolates *O. petiolata* from other species in its negative range, as it had a lower proportion of mandibular volume covered by such intervals (Fig.3d).

5.5 DISCUSSION

In this study, we aimed to investigate how mandible morphological variation influences stress patterns during biting and whether predatory ants exhibit enhanced biting performance compared to omnivorous species. We simulated the mechanical behavior of mandibles from 25 ant species belonging to different feeding habits, including predatory, omnivorous, and leaf-cutting species, and tested four biting scenarios. Bite simulations revealed no apparent specialization in biting performance between predatory and omnivorous species, and differences in stress patterns are more pronounced at the interspecific level than between feeding habits. Similar stress patterns were observed among species of all feeding habits, contrary to our initial expectation that predatory ants would exhibit superior performance compared to omnivorous species. This general pattern was reflected in the distribution of stress intervals along the mandibular volume, where groups of predatory and omnivorous species were not observed on the PCAs of all biting scenarios. The only exception to this general pattern was observed in the specialized predators (trap-jaw ants), which were generally isolated from other species on the PCAs. These ants typically had a larger volume of their mandibles filled with intervals of intermediate to high stresses.

Although there were no evident differences in stress patterns when comparing the differential use of the masticatory margin (entire or apical tooth only), we observed differences between pressure and strike biting. Pressure biting tended to generate higher relative stress levels along a larger mandibular surface area than strike bite, suggesting that pressing with mandibles is more mechanically demanding than striking. However, pressure biting tends to generate lower values of non-normalized stress than striking bite. Therefore, the mechanical demands of pressure biting are more relevant in long-lasting biting, where tiny failures can accumulate along the cuticle than in an acute high-stress peak, which is characteristic of strike biting. In some species, such as *A*. *aspersus*, *E*. *edentatum*, *O*. *petiolata*, *H*. *striatula*, and the major worker of *E*. *burchellii*, there were only subtle differences in stress distribution between pressure and strike biting, indicating a lack of specialization towards a specific biting behavior.

The form-function relationship of mandibles has been explored with FEA in other insect groups, such as Odonata (BLANKE et al., 2017) and beetles (HÖRNSCHEMEYER et al., 2013; GOYENS et al., 2014; 2015; 2016; KUNDANATI et al., 2020). Regarding ants, the relationship between mandible morphology and feeding habit is supported by data from fossil (BARDEN et al., 2020; LATTKE; MELO, 2020) and current lineages (CAMARGO et al., 2015; 2016; CHOWDHURY; RASTOGI, 2021; RICHTER et al., 2023). Studies focused on ant functional groups have found relationships between mandible morphology and species feeding habits (SILVA; BRANDÃO 2010; GIBB et al., 2015; SOSIAK et al., 2022). However, not all studies have found clear correlations. A recent study using a geometric morphometric approach with 3D data of the diverse ant genus *Pheidole* failed to find significant relationships between the mandible and head shape with feeding and nesting habits (Casadei-Ferreira et al. 2021). A phylogenetically broader assessment suggested that predatory ants tend to have longer and straighter mandibles, with predatory and omnivorous species differing in general morphological traits, although this effect was not statistically significant (SOSIAK; BARDEN, 2021). Also, the mandible length of more than 400 ant species showed no correlation with trophic position (DRAGER et al., 2023). Despite such reports, studies investigating the effects of mandible morphology on biting behavior in ants are still limited. Although there is evidence that mandible shape has an impact on the responses of these structures to bite-loading demands, these studies have often relied on small sets of species (LARABEE et al., 2018; KLUNK et al., 2021; WANG et al., 2022). In this context, our study stands out by comparing a higher number of species, and indicate that the main feeding habits, such as omnivory versus predation, are not good predictors of stress patterns in ant worker mandibles when considering only the variation in mandible shape and thickness.

Among the species studied, those with stouter mandibles, such as *C. pusillus* and majors of *Carebara* sp.01 and *P. aper*, are among the ones exhibiting relatively lower stress levels in pressure biting, as predicted. Interestingly, however, these stouter mandibles also showed relatively lower stresses in strike biting, as confirmed when considering normalized and non-normalized stress levels. Those species had mandibles with a broader blade and a subtle constriction near the mandibular base, a more convex curvature on the mandibular external face, and either short teeth or the absence of such structures. The lack of teeth means that the stresses generated in the masticatory margin do not concentrate heavily on smaller regions, as when teeth are present, but spread more evenly along the masticatory margin. A prominent mandibular curvature hampers the stress dissipation towards the delicate mandible blade, generating stress concentrations on the more robust masticatory margin and mandible base. *Pheidole* major workers are task specialists, often recruited for food processing and defense (WILSON, 1984; 2003; MERTL; TRANIELLO, 2009), behaviors that require strong bite forces and generate higher mechanical demands than other activities. A better biting performance of *Pheidole* major worker mandibles was previously highlighted (HUANG, 2012), mainly in pressure biting (KLUNK et al., 2021), but not in comparison with other ant genera. Majors of *Carebara* spp. perform similar specialized tasks as those described for *Pheidole* (KHALIFE; PEETERS 2020; KHALIFE et al., 2022), and their mandibular mechanical responses to bite-loading demands were correspondingly similar. Finally, species of *Cephalotes* spp. are known by their thicker exoskeleton, culminating in the morphological specialization of major heads to protect nest entrances (POWELL, 2008; 2009), but the mechanical demands of bite in worker mandibles were never tested before.

Two closely related species, *E. edentatum* and *H. striatula*, have similar mandibular morphology but show contrasting stress patterns in all biting scenarios tested, with *Holcoponera striatula* exhibiting relatively higher stress levels than *E. edentatum*. Regarding their positioning in the PCAs, both mandibles are more separated along the PC1 in simulations with the masticatory margin, suggesting that this mandibular region could represent an important source of mechanical distinction between the species. In fact, the masticatory margin in *E. edentatum* is wider (~0.9 mm) than in *H. striatula* (~0.3 mm), resulting in the bite load being applied along a broader area and hence dissipating over a larger area. However, a deeper investigation is needed to unveil the possible morphological aspects that explain the observed disparity in mechanical performance.

The trap-jaw ants, including *A*. *brevicornis*, *O*. *chelifer*, and *S*. *denticulata*, along with the major worker of *E*. *burchellii*, possess specialized mandibles, which in general are long, slender, with well-developed teeth, although the major worker of *E*. *burchellii* differed from the trap-jaw ants in having a hook-shaped mandible with a single tooth. Despite sharing these mandibular characteristics, those four mandibles displayed distinct stress patterns. *Strumigenys denticulata* and the major worker of *E*. *burchellii* showed proportionally higher stress levels throughout the mandibles than *A*. *brevicornis* and *O*. *chelifer*. However, when considering the non-normalized stress intervals, all these mandibles were highlighted on the PCAs by showing a proportionally larger volume filled with higher stress intervals, particularly in strike biting. These results do not support the initial hypothesis that trap-jaw ants would show relatively lower stress levels in strike biting. This partial contrast between the results of color maps and stress intervals provides intriguing suggestions about how trap-jaws deal with the mechanical demands of biting. Biting simulations demonstrate that trap-jaw mandibles tend to concentrate high-stress levels on the mandibular articulations, particularly for *A*. *brevicornis* and *O*. *chelifer*. Therefore, the long and slender mandibular blade withstands much lower stresses, reducing the risk of failure. Their general morphology and cross-section geometry do not suggest that their mandibles are prone to deform and withstand stress, such as observed in the snap-jaw ant *Mystrium camillae* (LARABEE et al., 2018), but this requires further investigation. Evidence from *Odontomachus monticola* highlights the importance of mandibular hollowness in withstanding the stresses generated by their powerful strike bites (WANG et al., 2022). The natural hollowness of this species appears to optimize the trade-off between power generation and impact resistance, compared to mandibles with higher indices of hollowness or completely solid mandibles (WANG et al., 2022). Accordingly, for all species tested in our study, their natural hollowness and cuticle thickness was kept for the simulations.

Although this study has shown some effects of mandible shape variation on stress distribution, it is important to note that the approach here presented does not fully capture the complexity of the mandible cuticle’s material properties. Insects have a gradient of material properties along the layers of their cuticle (RAJABI et al., 2017), which varies across different body regions. This material gradient proved to be functionally significant in some studies (DAS et al., 2018; JAFARPOUR et al., 2020; MATSUMURA et al., 2020) and is particularly relevant to ant mandibles, which can accumulate heavy metals along their masticatory margin, leading to increased cuticular stiffness in those regions

(SCHOFIELD et al., 2002; 2003; 2021; BROOMELL et al., 2008; POLIDORI et al., 2020). However, information about the variation in material properties of ant mandibles is still limited, with only a few studies restricted to a few leaf-cutting ants (SCHOFIELD et al., 2002; BRITO et al., 2017). Moreover, incorporating material property variation in FEA increases the simulation complexity and can lead to confounding effects on structure shape and material properties (RAYFIELD et al., 2007). Therefore, it is essential to consider the influence of material property variation under specific hypotheses and with appropriate data on the species in question. Further efforts are needed to measure the heterogeneity of cuticle material properties in ant mandibles to provide a better picture of their mechanical behavior.

Ant mandibles are essential for food capture and processing but also play a crucial role in other colony tasks performed by workers. Therefore, in some instances, mandible morphology could reflect the necessity to perform multiple tasks (ZHANG et al., 2020) or to excel in other activities, such as excavation (KHALIFE et al., 2018). The evolution of ant mandibles has been the subject of intense study in recent years (BOOHER et al., 2021; BOUDINOT et al., 2021; 2022; RICHTER et al., 2022), and although the shovel-shaped mandible is considered the Bauplan of extant ant mandibles (RICHTER et al., 2022), many extant ant lineages exhibit significant deviations from this general morphology (see also CHOWDHURY; RASTOGI, 2021). Our sampling set includes such variations and demonstrated relevant functional differences.

Biomechanical approaches such as FEA are essential for testing hypotheses about the role of mandible morphology in bite mechanics. Our results suggest that the main feeding habits of the ant species tested do not anticipate the general stress patterns observed in their mandibles under bite loading, and the observed interspecific morphological disparity have more relevant effects in biting performance. Additionally, more robust mandibles exhibited lower stress levels under both pressure and strike biting, whereas mandibles of trap-jaw ants did not show signs of adaptation to strike biting in relation to the remaining species. Further studies should explore the effects of variation in cuticle material properties, particularly considering the interspecific variation in heavy metal accumulation (POLIDORI et al., 2020). It would also be meaningful to investigate more widely the relationship between mandible morphology and bite force in ants (PÜFFEL et al., 2021; 2023).

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5.7 **Tables and figures**

Table 1. List of species considered for FEA in this study. Also shown are the worker type, energy and voxel size of the scans, surface area of the meshes (considered for load normalization), number of elements of each mesh, the applied load and the feeding habit of the species.

Species	**Worker type**	**Energy (keV)**	**Voxel size (µm)**	**Surface area (mm^2)**	**Number of elements**	**Applied load (N)**	**Feeding habit**
Acanthognathus brevicornis Smith, 1944	Normal	11	0.64193383	0.116002	1306962	0.0343	Specialized predator
Acromyrmex aspersus (Smith, 1858)	Normal	12	2.09	0.777902	645215	0.2300	Leaf-cutting
Azteca sp.	Normal	11	1.2828026	0.161742	545853	0.0478	Omnivorous
Bothroponera fugax (Forel, 1907)	Normal	10	1.25	0.265897	361389	0.0786	Generalized predator
Camponotus zenon Forel, 1912	Normal	12	2.45	0.463858	656142	0.1372	Omnivorous
Carebara sp.01	Major	20	2.44	1.191900	919446	0.3524	Generalized predator
	Normal	20	1.22	0.130098	445297	0.0385	
Cephalotes pusillus (Klug, 1824)	Normal	11	0.64193383	0.090715	428956	0.0268	Omnivorous
Dorymyrmex sp.	Normal	11	0.64193383	0.056881	501913	0.0168	Omnivorous
Eciton burchelli (Westwood, 1842)	Major	11	2.4	3.382100	1012354	1.0000	Generalized predator
	Mean	10	1.25	0.444433	755864	0.1314	
Ectatomma edentatum Roger, 1863	Normal	20	3.6667	2.132400	886718	0.6305	Generalized predator
Formica fusca Linnaeus, 1758	Normal	12	2.09	0.466144	876613	0.1378	Omnivorous
Heteroponera dentinodis (Mayr, 1887)	Normal	12	2.09	0.244014	781636	0.0721	Omnivorous
Holcoponera striatula (Mayr, 1884)	Normal	12	2.09	0.300672	175223	0.0889	Generalized predator
Lasius niger (Linnaeus, 1758)	Normal	12	2.09	0.234028	448285	0.0692	Omnivorous
Lenomyrmex foveolatus	Normal	11	0.64193383	0.066555	255517	0.0197	Generalized

Fernández & Palacio, 1999							predator
Lophomyrmex sp.01	Normal	20	2.44	0.254868	1153231	0.0754	Generalized predator
Myrmica ruginodis Nylander, 1846	Normal	12	1.31	0.142068	915314	0.0420	Generalized predator
Octostruma petiolata (Mayr, 1887)	Normal	12	2.09	0.100413	342483	0.0297	Generalized predator
Odontomachus chelifer (Latreille, 1802)	Normal	20	3.6667	2.821800	1123856	0.8343	Specialized predator
Parasyscia sp.	Normal	11	1.2	0.149357	618498	0.0442	Generalized predator
Pheidole aper Forel, 1912	Major	12	1.31	0.409701	861502	0.1211	Omnivorous
	Normal	12	0.66	0.090171	364652	0.0267	
Platythyrea cribrinodis (Gerstäcker, 1859)	Normal	11	2.4	1.269600	664280	0.3754	Generalized predator
Solenopsis sp.04	Normal	11	1.2	0.082008	282457	0.0242	Omnivorous
Strumigenys denticulata Mayr, 1887	Normal	12	1.22	0.086116	581390	0.0255	Specialized predator
Wasmannia affinis Santschi, 1929	Normal	12	0.66	0.024214	608544	0.0072	Omnivorous

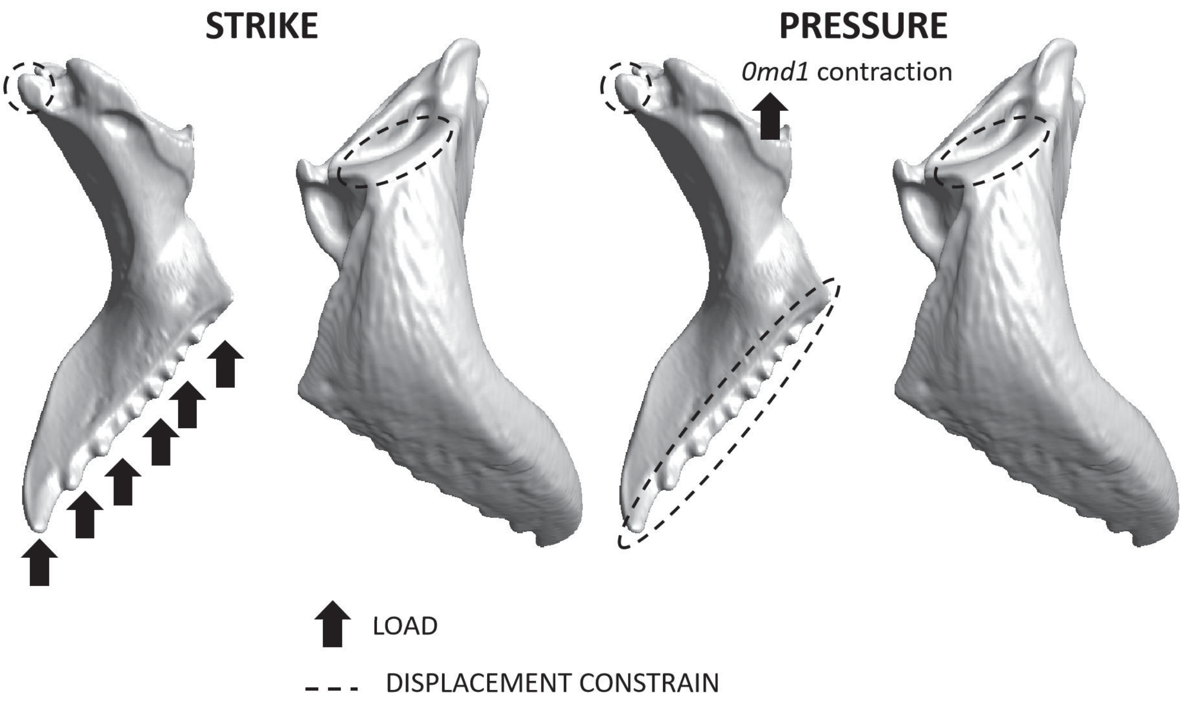

Fig.1. Diagrams depicting the boundary conditions for each biting scenarios. Highlighted are the conditions for strike and pressure biting with the entire masticatory margin.

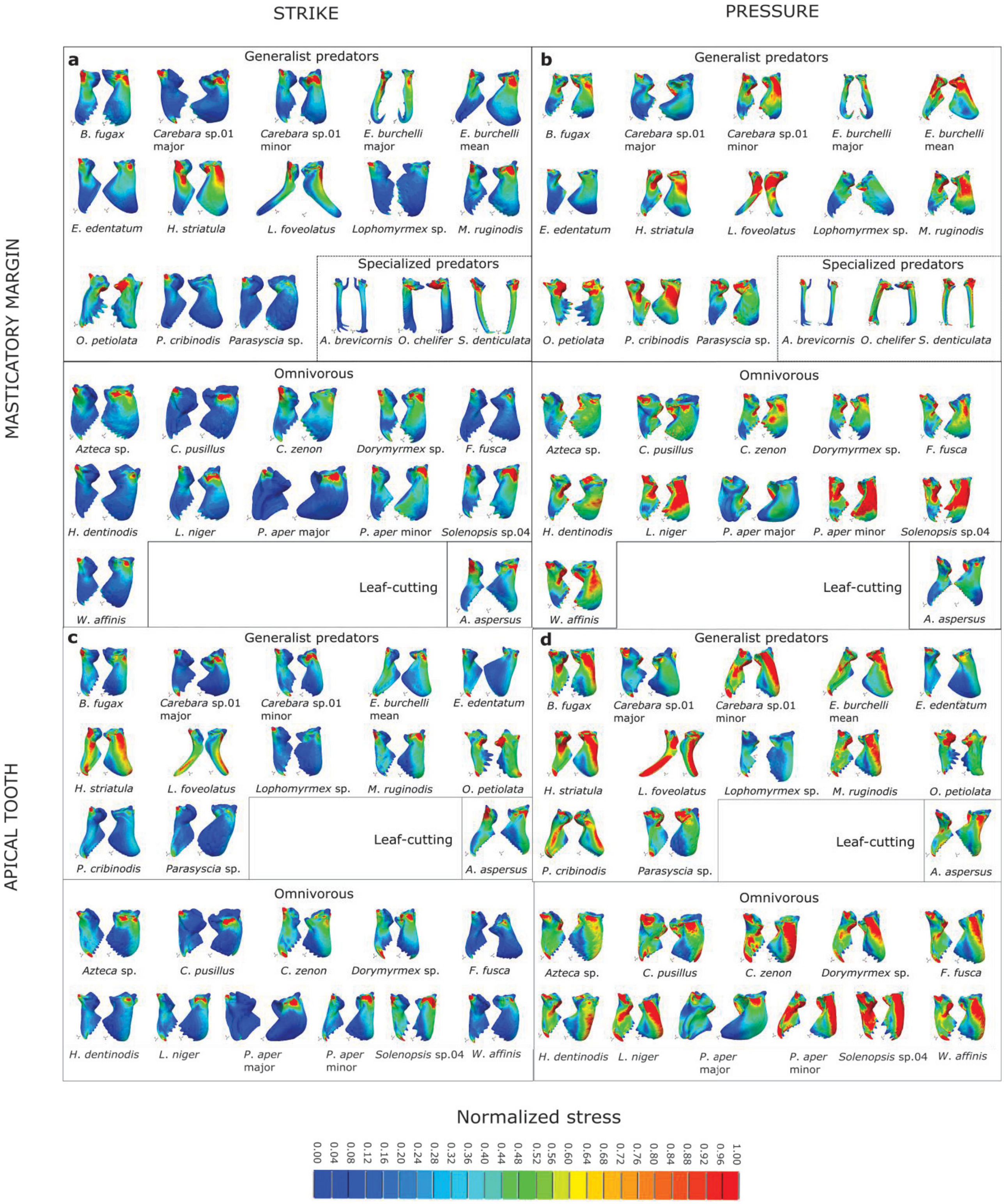

Fig.2. Color maps of FEA from all biting scenarios simulated. Species are grouped according to their main feeding habit. von Mises stress values were normalized based on a reference model to allow direct comparison between species and biting scenarios. Strike with the entire masticatory margin (a); Pressure with the entire masticatory margin; (c) Strike with the apical tooth; (d) Pressure with the apical tooth.

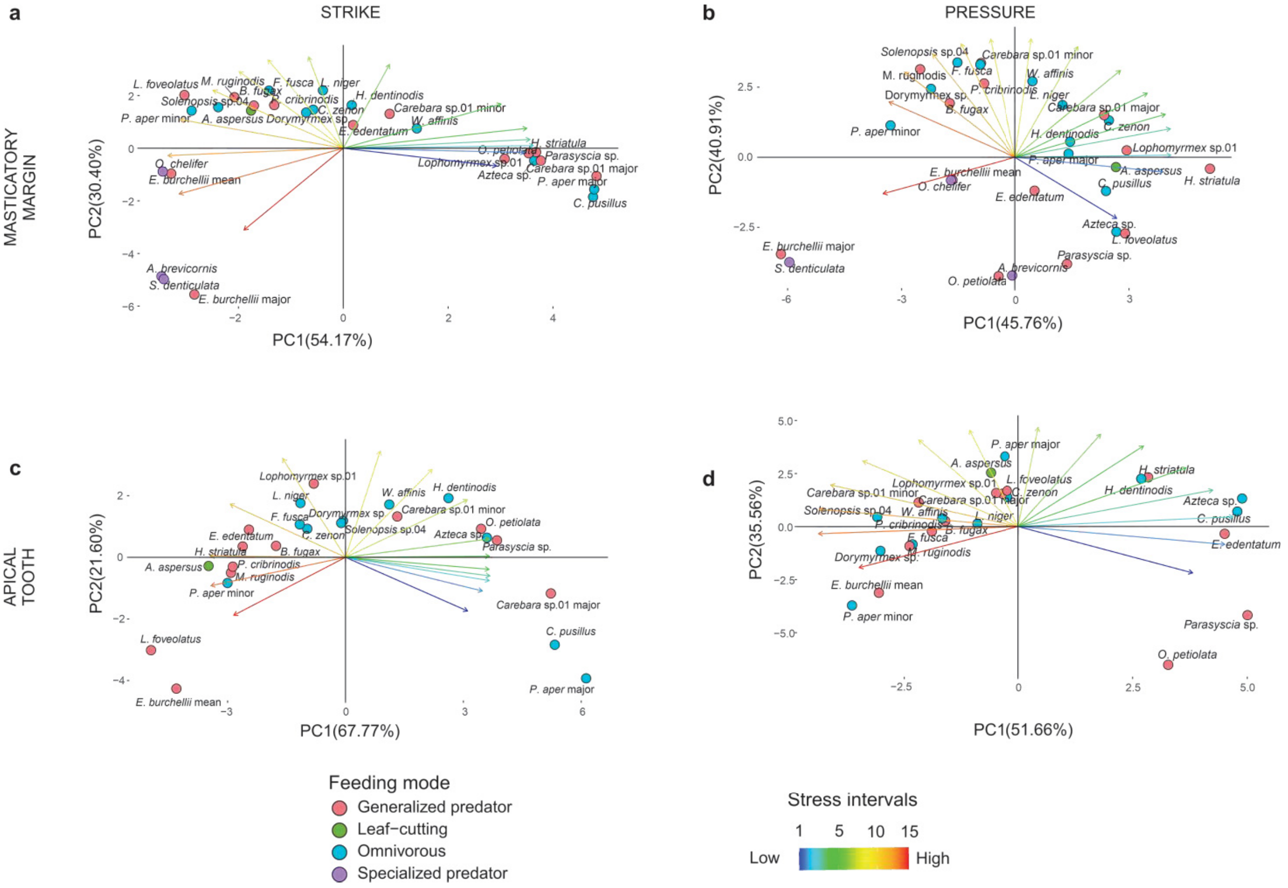

Fig.3. PCA based on the proportion of mandibular volume filled with each of the 15 stress intervals for all bite scenarios simulated: strike (a) and pressure (b) with the entire masticatory margin, strike (c) and pressure (d) with the apical tooth only. Colored circles depict the species main feeding habit, whereas colored arrows depict stress intervals, from the lowest stress value (blue - 1) toward the highest (red - 15).

6. **MANUSCRIPT 5**

Head endoskeleton as a strengthening tool to bite loading in ant (Hymenoptera: Formicidae) workers

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Cristian L. Klunk, Alexandre Casadei-Ferreira, and Roberto Keller conceived the ideas and designed methodology; Cristian L. Klunk, Alexandre Casadei-Ferreira and Evan Economo collected the data; Cristian L. Klunk and Marco A. Argenta analyzed the data; Cristian L. Klunk and Marcio R. Pie led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

6.1 Abstract

Mandibles are indispensable to ant activities, and the mandibular adductor muscles (0md1) are the most developed muscles of ant workers. Its contraction generates relevant mechanical demands in the head cuticle, where 0md1 fibers originate. The head capsule of ants also has internal cuticular structures suited for muscle insertion, but that could also be relevant as a structural support to the head capsule, like the head tentorium. Previous studies suggested that the three-dimensional shape of the head influences the amount of muscle fibers that ant heads can harbor. Also, plane head shapes of ant worker heads showed considerable variation in stress patterns under bite loading. However, how the 3D head shape and endoskeleton influence the mechanical response to biting load in ant heads was never tested. To improve our understanding of the head capsule's functional performance it is meaningful to investigate how the head cuticle deals with the demands of 0md1 contraction and which are the effects of the head tentorium in this context. We tested the effects of 0md1 contraction in volumetric models of *Pheidole minutula* Mayr 1878 major and minor worker heads, with and without the head tentorium, through Finite Element Analysis (FEA). We hypothesize that lower stress levels will be generated in the head capsule when the head tentorium is present. Since majors have much more powerful bites than minors, we expect them to show comparatively lower stress levels, irrespective of the head tentorium presence. As expected, the workers' head capsule showed relatively lower stress levels when the head tentorium was present, although this pattern was more evident for the major worker. Also, the major's head capsule showed relatively lower stress levels than the minor's head, irrespective of the presence or absence of the head tentorium. Such results demonstrate the relevance of the head tentorium in the head mechanical response under 0md1 contraction in both *Pheidole minutula* workers, and suggest that the head capsule of majors is adapted to deal with stronger bite-loading demands.

6.2 INTRODUCTION

Insect heads contain many structures and tissues associated with sensory systems and food processing. Eyes and antennae, essential sensory organs for most insects, are located in the head, along with the appendices related to food processing, like the mandibles, maxillae, and labium (SNODGRASS, 1935). The head interior also possesses several cuticular ridges that reinforce the head capsule, and a structure that represents the internal skeleton of insect heads called the head tentorium (SNODGRASS, 1935). The head tentorium is a relevant structure to muscle attachment, mainly to muscles associated with the pharynx and antennae in ants (KUBOTA et al., 2019; RICHTER et al., 2019; 2020; 2021; 2022; 2023; YAMADA et al., 2020; BOUDINOT et al., 2021). In addition, those internal cuticular structures proved to be relevant in providing mechanical support to the head capsule in some lineages of chewing insects (BLANKE et al., 2017a; 2018). In ants, the morphology of the head tentorium can vary between lineages and even among workers of the same species, especially regarding the relative development of some tentorial regions associated with muscle attachment.

In the genus *Pheidole*, there are two recognized worker types, the major and minor workers. Majors are larger and possess a disproportionately larger head than minors (HUANG, 2012; PIE; TRANIELLO, 2007; PIE; TSCHÁ, 2013; LILLICO-OUACHOUR et al., 2018). Beyond those morphological contrasts, *Pheidole* workers also show distinct roles in the colony. Minor workers are responsible for most of the colony’s non-reproductive tasks. They take care of the brood and the queen, forage, and ensure the nest maintenance. Majors, otherwise, are recruited mainly for defense and food processing (WILSON, 1984; MERTL; TRANIELLO, 2009; MERTL et al., 2010), even though they can perform other colony tasks in a less effective way (WILSON, 1984). The morphological evolution of *Pheidole* workers was investigated under many distinct approaches and related to different ecological aspects of their life histories. Evidence gathered so far suggests that the morphological evolution of *Pheidole* workers is not associated with feeding or nesting habits, and that size is a relevant intrinsic aspect of the observed worker morphological discrepancies (PIE; TRANIELLO, 2007; MOREAU, 2008; PIE; TSCHÁ, 2013; HOLLEY et al., 2016; SARNAT et al., 2017; FRIEDMAN et al., 2019; 2020; CASADEI-FERREIRA et al., 2021; 2022).

Food processing and defense usually demand strong bite forces, which agrees with the increased head size of majors (HUANG, 2012). Apart from the differences in head size, *Pheidole* majors generally have a heart-shaped head, which seems to be a morphological adaptation to harbor more 0md1 fibers (PÜFFEL et al., 2021; 2023a; 2023b, PAUL; GRONENBERG, 1999; PAUL, 2001). Also, there is evidence that plane shapes of *Pheidole* major heads are better suited to dissipate stress along a larger area instead of concentrating it on more restricted regions, as happens on most plane head shapes of minors (KLUNK et al., 2023). However, so far there were no attempts to investigate the effect of the three-dimensional variation in head morphology among *Pheidole* workers under bite loading, nor any effort to examine possible differential effects of the head tentorium in this mechanical context. Given the prominence of the 0md1 in ant workers, its contraction can substantially impact the head capsule cuticle, generating high levels of deformation and stress to be supported. The volume of the 0md1 is directly related to the biting capacity of insects (BLANKE, 2019; RÜHR et al., 2022), so that larger muscles result in higher loading demands on the head capsule. Moreover, stronger bites imply higher mandibular reaction forces, which also impact the head capsule through the mandibular articulations with the head. Therefore, besides packing the mandibular closing muscles, the head capsule should withstand the mechanical demands of muscle contraction and the associated mandibular reaction forces.

To test for the effects of the tentorium presence in the head capsule under the loading demands of the 0md1 contraction, we applied Finite Element Analysis (FEA) in volumetric representations of *Pheidole minutula* Mayr, 1878 worker heads. We hypothesize that in the presence of the tentorium, the head capsule will show proportionally lower stress levels since the head tentorium will act as a stress concentrator, hampering the spread of stresses towards the head capsule. Alternatively, a lack of an effect of the head tentorium would suggest that the primary axis of loading is different from the axis where the tentorium inserts in the head capsule, being its presence irrelevant to the mechanical demands associated with the 0md1 contraction. Moreover, we hypothesize that the morphology of the head capsule will also affect stress patterns, irrespective of the tentorium presence. Therefore, we speculate that the head of the major worker will show proportionally lower stress levels, being adapted to deal with more powerful bites, which agrees with its predominant roles in the colony.

6.3 MATERIAL AND METHODS

6.3.1 *Species*

To investigate the mechanical role of the head capsule and tentorium under the loading demands of the 0md1 contraction, we chose workers of *Pheidole minutula*, which is a South American ant species distributed mainly along the Amazon rainforest (GUÉNARD et al., 2017; JANICKI et al., 2016). As its name denotes, it is a small species of ant, with a poorly known biology. Relevant to our purposes, the head surface of both majors and minors is mostly smooth, with majors showing some sculpturing pattern on their head dorsal face (WILSON, 2003). This characteristic is important because complex sculpturing patterns are hard to be represented with the finite element meshes.

6.3.2 *Head Scanning*

One specimen of each *Pheidole minutula* worker type was scanned with a ZEISS Xradia 510 Versa X-ray micro-CT scanner at the Okinawa Institute of Science and Technology, using the software ZEISS Scout and Scan Control System. The exposure time for each specimen varied from 3 to 5 s, under a 4x objective and an ‘air’ filter. We set across the whole specimen a voltage of 40kV, 3W of power, under a ‘normal’ field mode and intensity levels of 15,000 and 17,000. Scan time varied from 30 to 50 min, which generated 801 projections from full 360º rotations.

6.3.3 *Head segmentation and reconstruction*

To reconstruct the worker heads we employed the software XMReconstructor, and for head segmentation, we used 3Dslicer v.5.2.2 (FEDOROV et al., 2012). To simplify the models, we used the software Blender 2.83 ®. Given the general morphological symmetry of the head capsule, we cut the volumetric models in the vertical plane along their middle line and considered only one half for the FEA. In this way, we were able to reduce the simulation computational demands, along with reducing the error caused by small asymmetries on the head sides, which are exacerbated by asymmetries resulting from the segmentation and mesh generation processes, as well as the consequent asymmetries in the definition of boundary conditions. We adopted similar mesh densities for each pair of head models (with and without the head tentorium), although allowing for substantial variation in mesh density between the different specimens due to the general differences in head morphology and size. Given that our intention here was solely to compare the stress patterns between head shapes and treatments in a comparative and relative manner, there is no need to perform mesh convergence tests, which are essential when the actual stress values are of interest (MARCÉ-NOGUÉ et al., 2015). Meshes were reduced in the Finite element solver FEBio 1.9.0 (MAAS ET AL., 2012).

6.3.4 *Finite Element Analysis*

To simulate the 0md1 contraction, we defined nodal loads on the internal head capsule, approximating the distribution of muscle fibers generally observed in ant heads (PAUL; GRONENBERG, 2002; LILLICO-OUACHOUR et al., 2018; RICHTER et al., 2019; 2020; 2021; BOUDINOT et al., 2021; KATZKE et al., 2022). To approximate the angle of muscle fiber, we considered that the 0md1 was composed of five leading muscular bundles, originating in five distinct head regions and inserted in the mandibular apodeme. One node of each area of muscular origin and one node on the apodeme were used as proxies to determine the load orientation of the respective muscular bundles (Fig.1a). We applied a 0.1N load on each head model equally distributed among the loaded nodes. To simulate the mandible reaction forces we constrained nodal displacement in all directions on the regions of mandibular articulation (VMA and DMA) with the head (Fig.1b). To maintain the head capsule in its position during biting, we also restricted in all directions the nodal displacements on the nuchal collar (Fig.1c). Finally, to account for the effects of the non-modeled half of the head, we also restricted the displacement on the nodes composing the cut region, only in the direction perpendicular to the cutting plane. In doing so, we avoid the simulation of unrealistic deformations caused by the absence of the other half of the head. Given that our focus was on the effects of the presence of the tentorium and the general head shape, we applied the same material properties to the cuticle of all modeled heads, including the tentorium. We modeled the cuticle as an isotropic and elastic material, with Young's modulus of 2.75 GPa, based on measurements of a leaf-cutting ant mandible (BRITO et al. 2017), and a Poisson's ratio as 0.3 (LARABEE et al., 2018; ZHANG et al., 2020; KLUNK et al., 2021; WANG et al., 2022). Simulations were conducted in the software FEBio 1.9.0 (MAAS et al., 2012).

6.4 RESULTS

Head tentorium affected stress distribution of both workers' head, albeit its effects were more prominent in the major worker. In simulations without the head tentorium, the head capsule of majors showed higher stress levels, especially in its dorsal and lateral regions (Fig.2a and c). Moreover, in the absence of the tentorium there was a considerable increase in stress levels on the head postgenal ridge (*pgr*) (Fig.2a and c). In minor workers, the differences in stress patterns according to the presence or not of the tentorium were less evident. There was a slight increase in stress levels when the tentorium was absent, mainly in the lateral and ventral walls of the head (Fig.2b and d). Contrary to the observed in the simulations with the major worker head, in the minor worker there was no relevant difference in stress levels in the *pgr* between the simulations (Fig.2b and d). In both workers' tentorium, stress concentrates on the upper portion of the anterior tentorial arm, whereas most of the remaining regions showed relatively lower stress levels (Fig.2a and b). Another relevant internal structure of the head that is affected by the presence of the head tentorium is the clypeal inflection (*cli*), a cuticular invagination of the clypeus. Without the head tentorium, the *cli* showed proportionally higher stress levels in both workers (Fig.3).

6.5 DISCUSSION

Our simulations showed that the head endoskeleton, especially the head tentorium, is a relevant structure that provides support to the head capsule, acting as a stress concentrator under the mechanical demands associated with the contraction of the 0md1, decreasing the amount of stress on the head capsule when present, in both worker types. However, its effects were more prominent in the head of the major worker, where considerably higher stress levels concentrate on the tentorium in relation to the observed on the head of the minor worker. Although the head capsule seems to be more affected by the presence of the tentorium, other internal cuticular elements showed differences in stress distribution in the scenarios tested, namely the postgenal ridge and the clypeal inflection. In the absence of the head tentorium, those structures tended to concentrate proportionally higher stress levels in both workers, except for the postgenal ridge in the minor worker, whose difference was only subtle. Moreover, the major worker head showed proportionally lower stress levels than the minor worker head, independently of the presence of the head tentorium, suggesting that the head external morphology is also a relevant variable in the mechanical response to the contraction of the adductor muscles.

The importance of the head endoskeleton in bite mechanics was already suggested for basal groups of chewing insects (BLANKE et al., 2017*a*; 2018). Such studies demonstrated that the interior of the head capsule possesses a set of ridges and cuticular invaginations that act as a system that concentrates strains, resulting in a reinforcement of the head capsule under the mechanical demands generated by the mandibular closing muscles contraction. The efficiency of this reinforcement system was tested on specimens of distinct insect orders, which have disparate head morphologies and mandibular orientations, suggesting that a set of cuticular structures are essential to provide the necessary protection to bite-loading demands in a diverse range of insect lineages (BLANKE et al., 2017a; 2018). Our approach, on the other hand, showed that there could be a variation in the relevance of the head tentorium among different workers of the same ant species. Moreover, by applying simulations in head capsules with and without the head tentorium, we could demonstrate which head regions would be in more demand without this structure, like the clypeus inflection, the postgenal ridge, and the head capsule.

Interestingly, our results suggest that the major worker head tentorium concentrates proportionally higher stress levels than the minor worker, which helps to explain why the major worker head capsule was less stressed than the minor head capsule. The morphology of the head tentorium can vary among different ant species (KUBOTA et al., 2019; RICHTER et al., 2019; 2020; 2021; 2022; 2023; YAMADA et al., 2020; BOUDINOT et al., 2021), but although there are morphological differences in the head tentorium of *P*. *minutula* workers, such variation is unlikely related to the distinct stress patterns observed. In the major worker the corpotendon (KUBOTA et al., 2019) is shorter, and the internal and external plates (KUBOTA et al., 2019) are also relatively less developed than in the minor worker tentorium. Such regions are not heavily stressed in both workers, being important regions of muscle attachment (KUBOTA et al., 2019; RICHTER et al., 2019; 2020; 2021; 2022; 2023; YAMADA et al., 2020; BOUDINOT et al., 2021). Therefore, the impact of such morphological distinctions is more related to the disproportional increase of the mandibular adductor muscles to the detriment of other muscles and internal tissues of *Pheidole* majors' head (LILLICO-OUACHOUR et al., 2018), than to direct mechanical effects on bite loading demands. A relevant drawback of our approach is that we were unable to accurately represent the morphology of the minor worker tentorium. Given its small size, the scanning resolution does not allow us to capture the hollowness of the tentorium arms, and such hollowness proved to be relevant in the biomechanical context (WANG et al., 2022), given its effect on structure deformation capabilities.

Differences in stress pattern between worker heads agree with the observed division of labor of *Pheidole* species. Irrespective of the presence or not of the head tentorium, the head of the major worker showed relatively lower stress levels than the head of the minor worker. It suggests that such a head morphology is adapted to deal with more powerful bites. In *Pheidole* colonies, majors are particularly recruited to act on food processing and defense (WILSON, 1984; MERTL; TRANIELLO, 2009; MERTL et al., 2010), behaviors that usually demand stronger bites. The disproportionately larger mandibular closing muscles seen in *Pheidole* majors (LILLICO-OUACHOUR et al., 2018), and the stronger bites of majors in relation to minors (HUANG, 2012), corroborate the idea that those individuals are adapted to perform powerful bites. Therefore, our results suggest that besides contributing to storing larger muscles (PÜFFEL et al., 2021; 2023a; 2023b, PAUL; GRONENBERG, 1999; PAUL, 2001), the morphology of major worker heads is also tuned to improve its mechanical responses to bite-loading demands.

The ant head tentorium and internal cuticular ridges proved to be relevant structures that reinforce the head capsule against the mechanical demands of bite, delivered essentially by the contraction of the mandibular closing muscles and the mandibular reaction forces transmitted by the mandibular articulations with the head. Moreover, differences in general head morphology, like the head capsule shape, also showed relevant impacts on stress patterns under this context. Our results agree with previous studies suggesting that majors of *Pheidole* have head shapes better suited to deal with bite-loading demands (KLUNK et al., 2023). Given the general stress patterns observed in the heads of *P. minutula* workers, it would be interesting to explore other aspects that could contribute to the mechanical responses of structures to different loading conditions, like the variation in material properties usually observed, for example, in insect mandibles (SCHOFIELD et al., 2002; 2003; 2021; BROOMELL et al., 2008; POLIDORI et al., 2020; REITER et al., 2023), but still poorly explored along the diversity of ant species, specially regarding ant heads.

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6.6 REFERENCES

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6.7 Figures

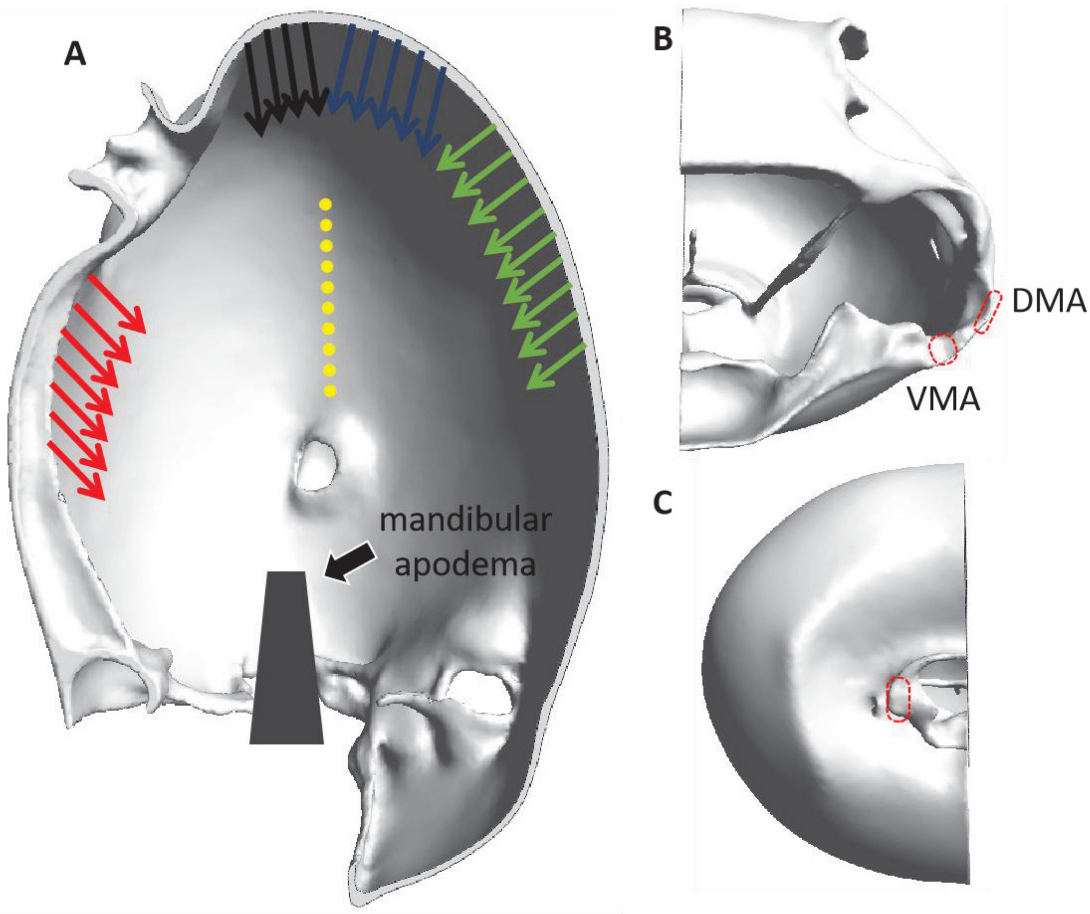

Fig.1. Load and displacement restrictions as defined for each FEA, represented by the half head of *Pheidole minutula* minor worker without the head tentorium. A: We projected the MAM as a five-bundled muscle to account for their main variation in muscle fiber orientation commonly found among ant and wasp heads. Each arrow and circle color represents a different muscle bundle with a distinct orientation. Yellow circles represent muscle fibers originating from the lateral wall of the head, pointing to the reader's view. For each muscle bundle, we extracted the coordinates of a node located in the center of nodal distribution as a reference to vector decomposition. The mandibular apodeme is represented in this schematic version of load orientation because it was used as a reference to determine the load direction for each muscle bundle. B: Red dashed ellipses highlight the regions of mandibular articulation, whose nodes were restricted to avoid nodal displacements in all directions and therefore simulate the instant of bite. C: Red dashed ellipses highlight the region of the nuchal collar whose nodes were restricted to avoid displacement in all directions and maintain the head position during bite simulation. DMA = Dorsal Mandibular Articulation. VMA = Ventral Mandibular Articulation.

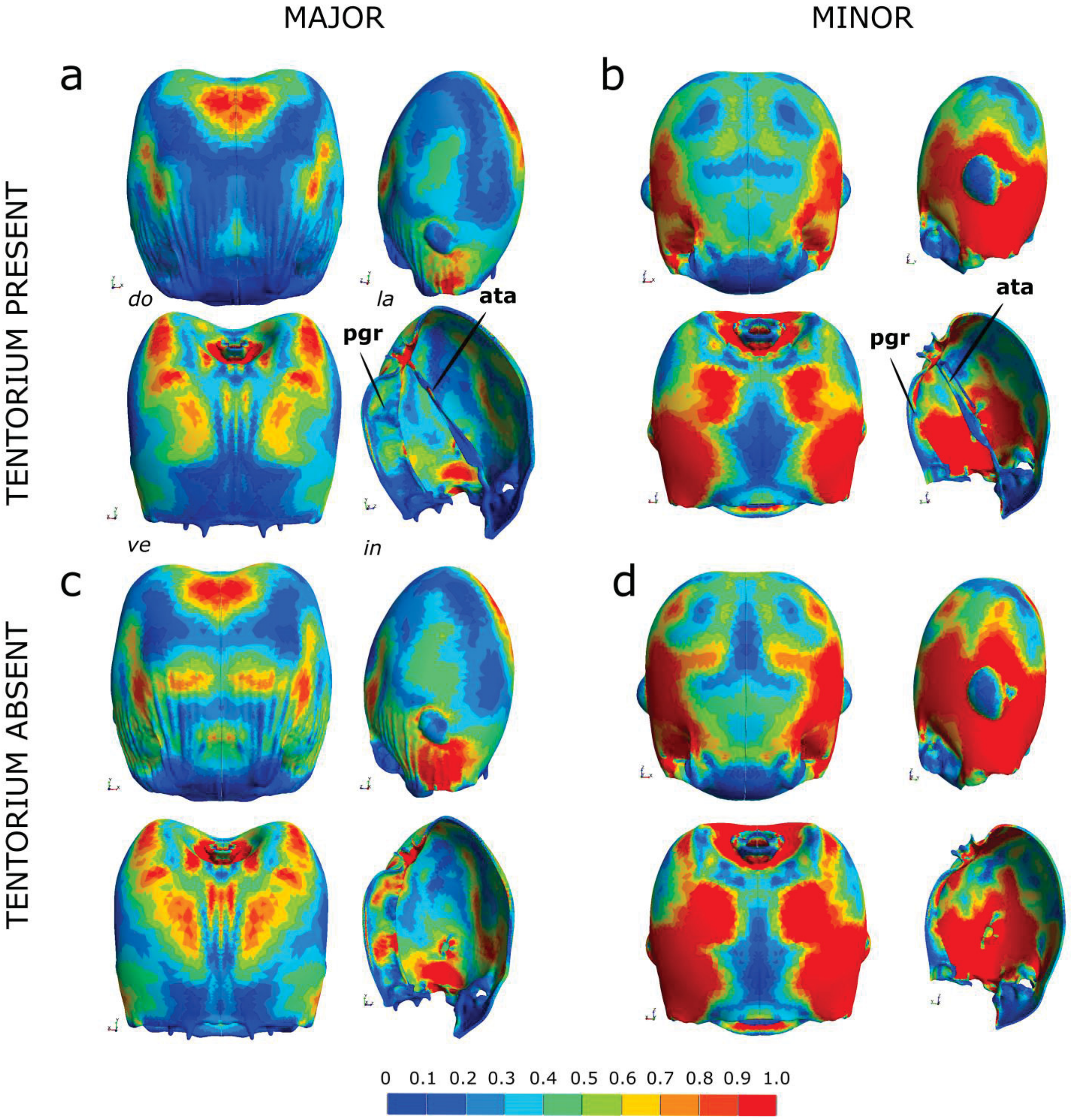

Fig.2. von Mises equivalent stress distribution on *Pheidole* major (a and c) and minor (b and d) worker heads, with (a and b) and without (c and d) the head tentorium, according to FEA simulations. Stress distribution was normalized according to a reference model to allow direct comparisons between simulations. *ata* = anterior tentorial arm; *pgr* = postgenal ridge. do = dorsal; la = lateral; ve = ventral; in = internal.

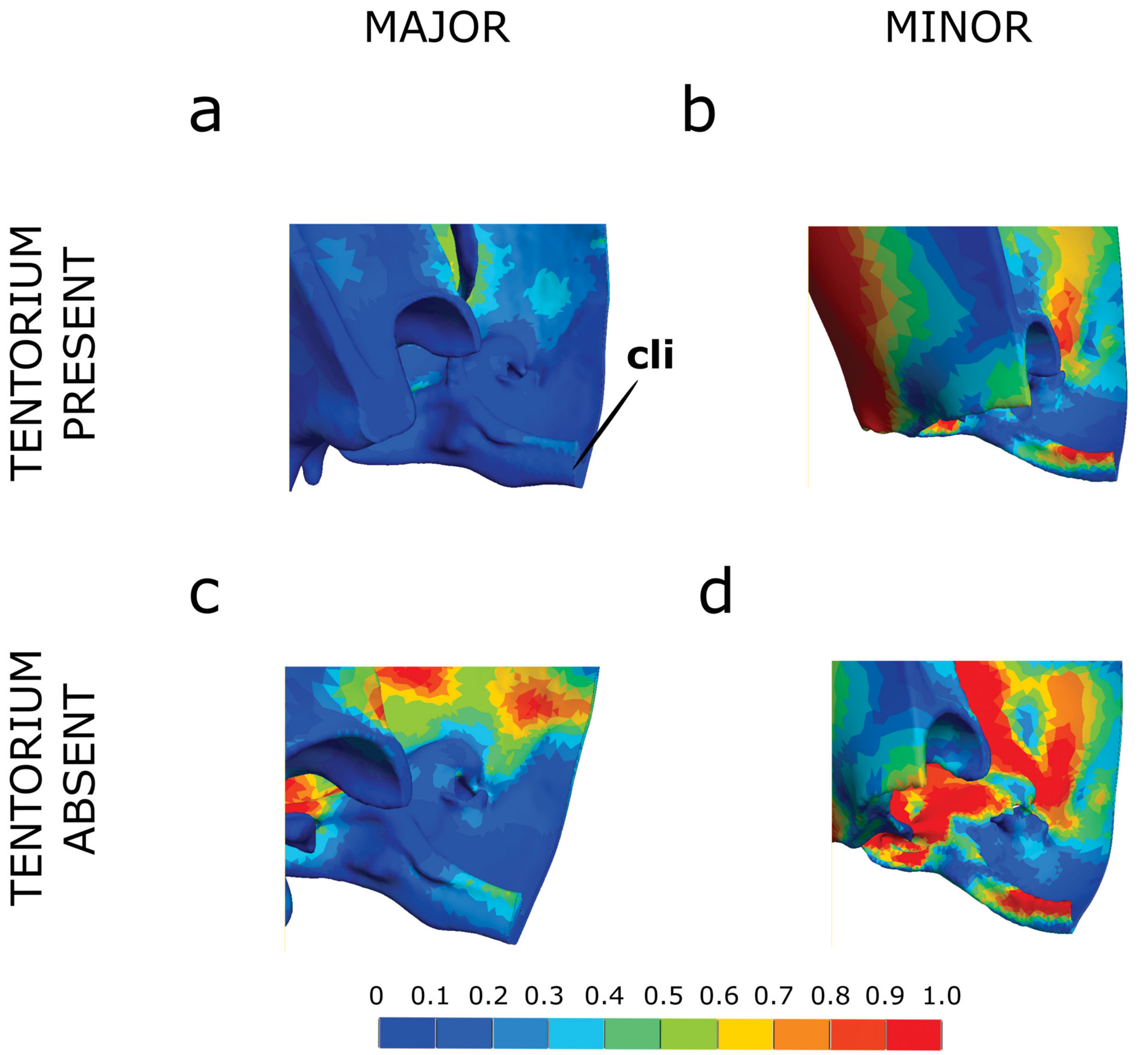

Fig.3. von Mises equivalent stress distribution on *Pheidole* major (a and c) and minor (b and d) worker heads, with (a and b) and without (c and d) the head tentorium, highlighting the concentration of stress on the clypeal inflection (*cli*).

7.1 CONSIDERAÇÕES FINAIS

Entre as mais de 14.000 espécies descritas até o momento (BOLTON, 2023), formigas se destacam pela sua impressionante diversidade morfológica, incluindo variações na forma da cabeça e mandíbulas (WHEELER, 1910). Operárias de formigas dependem das suas mandíbulas para desempenhar boa parte das atividades diárias da colônia, o que sugere uma perspectiva funcional na evolução dessa marcante diversidade morfológica. Aqui exploramos os efeitos da forma da cabeça e mandíbulas de operárias de formigas na resposta mecânica à mordida através de uma abordagem funcional. Especificamente, simulamos diferentes cenários de carregamento em reconstruções digitais de cabeças e mandíbulas de operárias de formigas para emular as demandas mecânicas da mordida, aplicando a Análise de Elementos Finitos (AEF) para aproximar as respostas mecânicas dessas estruturas.

Nossa abordagem focou nos níveis intra e interespecífico de variação morfológica em formigas. Ao nível intraespecífico, exploramos a variação morfológica entre operárias do gênero dimórfico *Pheidole*, cujas operárias maiores possuem cabeças alargadas e mandíbulas robustas (HUANG, 2012; PIE; TRANIELLO, 2007; PIE; TSCHÁ, 2013; LILLICO-OUACHOUR et al., 2018), usualmente empregadas para defesa e processamento de alimentos (WILSON, 1984; MERTL; TRANIELLO, 2009; MERTL et al., 2010). Ao longo dos manuscritos um, dois, quatro e cinco, sugerimos que a forma da cabeça e mandíbulas das operárias maiores de *Pheidole* estão melhor adaptadas para lidar com as demandas mecânicas da mordida em relação às operárias menores. Essas vantagens biomecânicas são esperadas com base na divisão de trabalho observada nas colônias do gênero no que concerne as principais tarefas executadas pelas operárias maiores, que demandam mordidas mais fortes. Investigamos os efeitos da variação morfológica da cabeça e mandíbulas de formigas ao nível interespecífico nos manuscritos três e quatro. Incorporando espécies de grande parte das subfamílias de formigas atualmente reconhecidas, pudemos testar potenciais adaptações funcionais na cabeça e mandíbulas de formigas, sugerindo alguns aspectos morfológicos que podem contribuir com um melhor desempenho mecânico sob as demandas da mordida.

Embora a presente tese tenha tido como objetivo aperfeiçoar nosso conhecimento acerca do desempenho mecânico da cabeça e mandíbulas de formigas, bem como fornecer perspectivas acerca da evolução morfológica do grupo, a abordagem aqui desenvolvida está longe de oferecer respostas definitivas, representando um passo importante para guiar futuros estudos acerca da mecânica da mordida de formigas. Ao longo dos cinco manuscritos dessa tese, discutimos acerca de várias regiões da cabeça e mandíbulas que usualmente são significativamente tensionadas durante a mordida. Nossa abordagem focou nos efeitos da variação morfológica (essencialmente forma da mandíbula e cabeça, além da espessura da cutícula em modelos 3D) na resposta mecânica à mordida. Porém, a AEF pode ser empregada para explorar os efeitos da variação intra e interespecífica nas propriedades materiais da cutícula. É notável que a cutícula não é um material homogêneo como considerado aqui, mas apresenta um gradiente de propriedades mecânicas funcionalmente relevante (DAS et al., 2018; JAFARPOUR et al., 2020; MATSUMURA et al., 2020). Além desse gradiente esperado pela natureza estratificada da cutícula, muitos artrópodes, incluindo formigas, são conhecidos por empregar metais pesados para aumentar a rigidez da cutícula dos apêndices utilizados para morder e perfurar (SCHOFIELD et al., 2002; 2003; 2021; BROOMELL et al., 2008; POLIDORI et al., 2020; REITER et al., 2023). Nesse sentido, estudos futuros poderão enriquecer nosso conhecimento da mecânica da mordida de formigas por considerar variações nas propriedades materiais da cutícula na AEF. Todavia, um passo prévio envolve aumentar nosso conhecimento acerca da variação nas propriedades materiais da cutícula de formigas, que atualmente está restrito à cutícula de algumas poucas espécies de formigas cortadeiras (SCHOFIELD et al., 2021; BRITO et al., 2017; LI et al., 2020). Propriedades mecânicas relevantes no contexto da AEF, como o módulo de Young, são desafiadoras para se medir, e a natureza dinâmica dos tecidos biológicos impede a aquisição de dados definitivos para as espécies. Dessa forma, essa informação será mais interessante para abordagens comparativas.

Outro ponto importante se refere a um maior conhecimento acerca da força de mordida de formigas. Embora alguns modelos teóricos vem sendo desenvolvidos para estimar a força de mordida com base apenas em informações morfológicas, como largura da cabeça (PÜFFEL et al., 2021; 2023a), combinado ou não com aspectos mecânicos como a velocidade de fechamento das mandíbulas (LARABEE et al., 2018), o uso de sensores para medir diretamente a força da mordida também representa uma avenida interessante à ser explorada (HUANG, 2012; HAO et al., 2018; RÜHR et al., 2022; PÜFFEL et al., 2023b). Dispositivos para medir diretamente a força da mordida de insetos, incluindo formigas, tem sido desenvolvidos e fornecendo medidas confiáveis (HUANG, 2012; HAO et al., 2018; RÜHR et al., 2022; PÜFFEL et al., 2023b). Incorporando força de mordida e propriedades materiais mais próximas da realidade na AEF vai permitir uma investigação mais ampla acerca de potenciais adaptações funcionais na cabeça e mandíbulas de operárias de formigas, separando os efeitos da morfologia e propriedades materiais sob demandas mecânicas mais realistas.

7.2 CONCLUSIONS

Among its more than 14,000 species described so far (BOLTON, 2023), ants draw attention due to their incredible morphological diversity, including variation in head and mandible shape (WHEELER, 1910). Ant workers rely on their mandibles to perform most of the daily colony tasks, which suggests a functional perspective on the evolution of such a remarkable morphological diversification. Here we explored the effects of the ant worker head and mandible shape in the mechanical responses to bite loading through a functional approach. Specifically, we simulated different loading scenarios in digital reconstructions of ant worker heads and mandibles to emulate the mechanical demands of bite, applying Finite Element Analysis (FEA) to approximate the mechanical responses of these structures.

Our approach focused on intra and interspecific levels of ant morphological variation. At the intraspecific level, we explored the morphological variation among workers of the dimorphic ant genus *Pheidole*, whose major workers have enlarged heads and robust mandibles (HUANG, 2012; PIE; TRANIELLO, 2007; PIE; TSCHÁ, 2013; LILLICO-OUACHOUR et al., 2018), usually employed to defend and process food (WILSON, 1984; MERTL; TRANIELLO, 2009; MERTL et al., 2010). Along manuscripts one, two, four, and five, we suggested that the head and mandible shape of *Pheidole* majors are better suited to deal with the mechanical demands of bite in relation to minors. Such biomechanical advantages are expected based on the colony division of labor and the main tasks that majors are supposed to perform, which are associated with more powerful bites. We investigated the effects of ant head and mandible morphological variation at the interspecific level in manuscripts three and four of the present thesis. By incorporating species from many of the currently recognized ant subfamilies, we tested for potential functional adaptations in ant worker head and mandible, suggesting some morphological aspects that contribute to better mechanical performance under bite demands.

Although intended to improve our knowledge about the functional performance of ant worker mandible and head and provide insights about the morphological evolution of ants, the approach developed in the present thesis is far from definitive, representing one step to guide future studies on ant bite mechanics. Along the five manuscripts of this thesis, we discussed several mandibular and head regions that usually are heavily stressed during biting. Our approach focused on the effects of the morphological variation (essentially head and mandible shape, and in 3D models, cuticle thickness) in the mechanical response to biting. However, FEA could also be employed to explore the effects of intra and interspecific variation in cuticular material properties. Notably, the cuticle is not a homogeneous material as considered here but shows gradients of mechanical properties that are functionally relevant (DAS, et al. 2018; JAFARPOUR, et al. 2020; MATSUMURA et al., 2020). Besides the material property gradients expected by the layered structure of the cuticle, several arthropods, including ants, are known to employ heavy metals to increase the stiffness of appendices used to bite or pierce (SCHOFIELD et al., 2002; 2003; 2021; BROOMELL et al., 2008; POLIDORI et al., 2020; REITER et al., 2023). In this sense, future studies could enrich our knowledge of ant bite mechanics by considering discrepancies in cuticle material properties in FEA. However, a previous and essential step would be to improve our knowledge of ant cuticle variation in material properties, which currently is restricted to the cuticle of a few leaf-cutting ants (SCHOFIELD et al., 2021; BRITO et al., 2017; LI et al., 2020). Mechanical properties relevant to FEA, such as Young's modulus, are challenging to measure, and the dynamic nature of a biological tissue prevents the acquisition of definitive species data. Therefore, such information would be more useful in a comparative sense.

Another relevant step is to achieve a better understanding of ant bite forces. Although several theoretical models have been developed to estimate bite forces only with morphological information, like head width (PÜFFEL et al., 2021; 2023a), combined or not with mechanical aspects like mandible closing speed (LARABEE et al., 2018), the use of sensors to directly measure ant bite force also represent an exciting avenue to explore (HUANG, 2012; HAO et al., 2018; RÜHR et al., 2022; PÜFFEL et al., 2023b). Mechanical devices to directly measure insect bite forces, including ants, have been successfully developed and provided reliable measurements (HUANG, 2012; HAO et al., 2018; RÜHR et al., 2022; PÜFFEL et al., 2023b). By incorporating reliable bite forces and cuticular material properties into FEA, it would be possible to investigate the potential for functional adaptations of ant worker heads and mandibles in a broader sense, taking apart the effects of morphology and material properties under meaningful loading demands.

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