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## **Mandibular morphology, task specialization, and bite mechanics in Pheidole ants (Hymenoptera: Formicidae) — Source link**

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1 **Mandibular morphology, task specialization, and bite mechanics in *Pheidole* ants**

2 (Hymenoptera: Formicidae)

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12  
13 **Abstract**

14 The remarkable ecological and evolutionary success of ants was associated with the evolution  
15 of reproductive division of labor, in which sterile workers perform most colony tasks whereas  
16 reproductives become specialized in reproduction. In some lineages, the worker force became  
17 further subdivided into morphologically distinct subcastes (e.g. minor vs. major workers),  
18 allowing for the differential performance of particular roles in the colony. However, the  
19 functional and ecological significance of morphological differences between subcastes is not  
20 well understood. Here, we applied Finite Element Analysis (FEA) to explore the functional  
21 differences between major and minor ant worker mandibles. Analyses were carried out on  
22 mandibles of two *Pheidole* species, a dimorphic ant genus. In particular, we test whether  
23 major mandibles evolved to minimize stress when compared to minors using combinations  
24 of tooth and masticatory margin bites under strike and pressure conditions. Majors performed  
25 better in pressure conditions yet, contrary to our expectations, minors performed better in  
26 strike bite scenarios. Moreover, we demonstrate that even small morphological differences  
27 in ant mandibles might lead to substantial differences in biomechanical responses to bite  
28 loading. These results also underscore the potential of FEA to uncover biomechanical  
29 consequences of morphological differences within and between ant worker castes.

30 **Key words:** Cuticle; Division of labor; Finite Element Analysis; Mandible; Trulleum;  
31 Worker polymorphism.

## 32                    **Introduction**

33                    The evolution of highly complex societies in ants was associated with the evolution  
34 of reproductive division of labor, in which sterile workers perform most quotidian colony  
35 tasks, whereas reproductives become specialized in colony reproduction (Wilson 1971,  
36 Hölldobler & Wilson 1990). These changes were accompanied by substantial morphological  
37 differences among reproductives and workers, with the latter giving up reproduction and  
38 dispersal capacities to allow for morphological adaptations that improve the ergonomic  
39 efficiency of the colony as a whole. In some ant lineages, the worker force became further  
40 subdivided into morphologically distinct subcastes (e.g. minor vs. major workers), and such  
41 differences are thought to allow for the differential performance of particular roles in the  
42 colony, such as seed milling and colony defense (Wilson 1953; Oster & Wilson, 1978). In  
43 ants, polymorphism evolved in several lineages, and its role to facilitate task specialization  
44 is widely recognized (Wilson 1953; Oster & Wilson, 1978; Wills et al., 2018), and the genetic  
45 (Gadagkar 1997; Huang et al., 2013), ecological (Powell & Franks, 2005; 2006; Powell 2009)  
46 and developmental (Wheeler 1991) determinants associated with the occurrence of worker  
47 polymorphism have been explored in several ant lineages (Wills et al., 2018).

48                    The genus *Pheidole* shows an interesting pattern among its almost 1,200 known  
49 species (Bolton 2020), the development of a dimorphic worker subcaste, represented by  
50 major and minor workers, where majors have a disproportionately larger head (Wilson 1953;  
51 2003). *Pheidole* species are distributed worldwide, but most of its diversity and abundance  
52 is concentrated in the tropics (Economo et al., 2015a; 2019). Although *Pheidole* species are  
53 typically considered diet generalists (Wilson 2003), some species might show degree of food  
54 preferences (Rosumek 2017). Of all their food items, feeding on seeds evolved many times  
55 in the genus and has been indicated as an important factor to explain the lineage  
56 diversification due to behavioral and morphological adaptations related to seed harvesting  
57 and processing (Moreau 2008). Since majors are specialized in tasks as colony defense and  
58 food processing (Wilson 1984; 2003), their larger heads could be a consequence of  
59 evolutionary pressures towards the specialization to those tasks (Pie & Traniello, 2007),  
60 although evidence gathered so far has been mixed (e.g. Holley et al., 2016).

61                    Understanding the main trends in the morphological evolution of *Pheidole* received  
62 considerable attention in the past decade, with different approaches being employed to

63 understand the evolution of a wide variety of structures, showing contrasting results to the  
64 relative contributions of size and shape to the morphological diversity of the genus (Pie &  
65 Traniello, 2007; Pie & Tschá, 2013; Sarnat et al., 2017; Friedman et al., 2019; 2020), yet  
66 little is known about the evolution of mandibular morphology in *Pheidole*. Ants have a  
67 typical pterygote mandible with two articulations with the head (Snodgrass 1935), the dorsal  
68 and ventral mandibular joints (Richter et al., 2019). The mandible is the main structure used  
69 to interact with the environment (e.g. biting, carrying, excavating, cutting, fighting) (Wheeler  
70 1910). Mandibular movement is led by two muscles, the *craniomandibularis internus (mci)*,  
71 whose contraction close the mandibles, and the *craniomandibularis externus (mce)*,  
72 responsible for the opening process (Snodgrass 1935; Richter et al., 2019). The *mci* fibers  
73 attach to the mandible through a mandibular cuticular projection called apodeme (Paul &  
74 Gronenberg, 1999). The angle of attachment to the apodeme, combined to the sarcomeres  
75 length, are directly related to the velocity and force of the mandibular movement (Paul &  
76 Gronenberg, 1999), so that *mci* is considered the key to the versatility of ant mandibles  
77 (Gronenberg et al., 1997; Paul 2001), being much more developed than the *mce* (Paul 2001).  
78 In *Pheidole* majors the *mci* is remarkably large, where its increase in size in relation to minors  
79 happens at the expense of the glandular, digestive, and nervous system in the head (Lillico-  
80 Ouachour et al., 2018). Fibers of the *mci* continue to develop even for days after the adult  
81 emergence in both subcastes, and this characteristic correlates to behavioral development in  
82 workers (Muscedere et al., 2011).

83         Regardless of the importance of mandibles to many aspects of ant life history, little  
84 is known about how the morphological variation between species or worker subcastes relates  
85 to bite loads, except for one specialized snap-jaw species (Larabee et al., 2018). Worker  
86 polymorphism can lead to behavioral specialization, mainly through variation in mandible  
87 morphology (Silva et al., 2016), but biomechanical approaches to directly assess this  
88 relationship in ants are scarce (Larabee et al., 2018). To understand how mandible  
89 morphology relates to the biomechanical demands of bite, it is important to employ  
90 approaches that allow for the direct assessment of bite loading conditions. Finite Element  
91 Analysis (FEA) is a numerical method that approximates the mechanical simulation of  
92 loading conditions in structures of interest. By applying loads and defining the boundary  
93 conditions (movement restrictions) on the structure, FEA estimates the mechanical response,

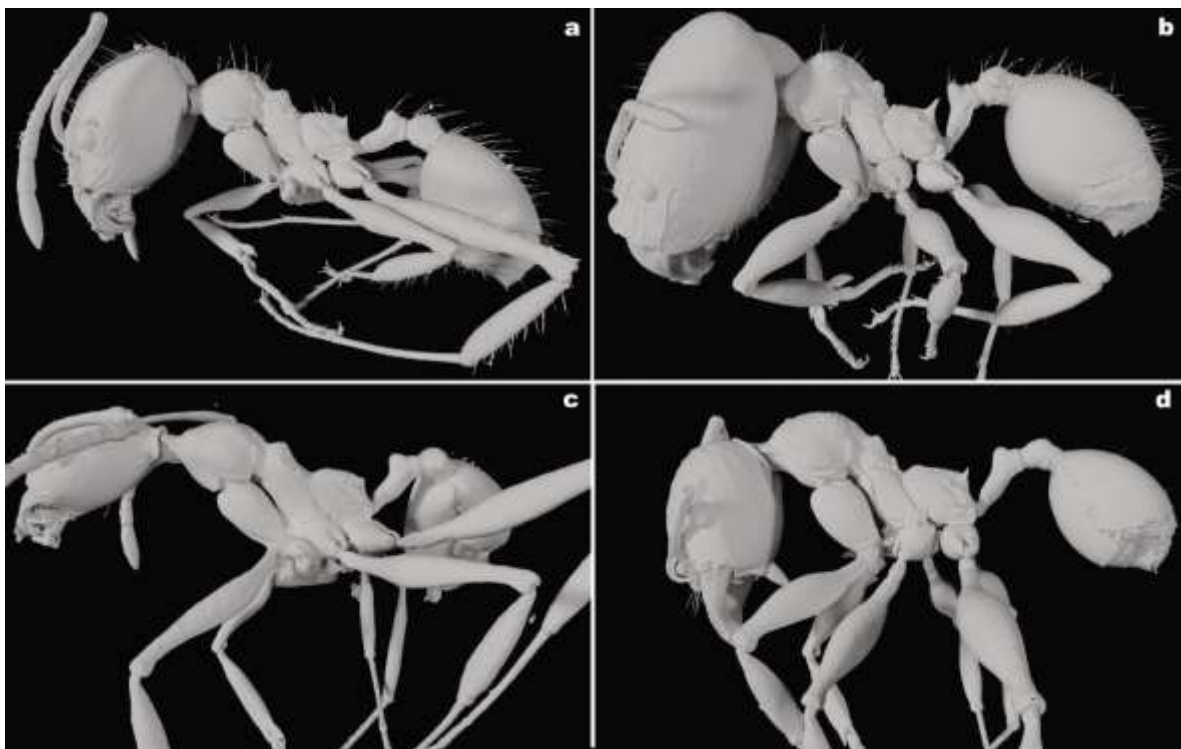
94 i.e., how stress flows along the structure according to its shape (Azevedo 2003; Rayfield  
95 2007). By employing FEA, one might assess how variation in mandibular morphology  
96 among ant species as well as between subcastes translates into the capacity of mandibles to  
97 deal with bite loading demands (Larabee et al., 2018), as also explored for the evolution of  
98 mandible form in dragonflies (Blanke et al., 2017) and stag beetles (Goyens et al., 2015;  
99 2016).

100           To improve our understanding of morphological evolution in *Pheidole* species, and  
101 the role of morphological differentiation to improve task specialization in polymorphic ants,  
102 we simulate several bite scenarios *in silico* by applying FEA (Azevedo 2003; Rayfield 2007)  
103 on 3D models of minor and major mandibles of two *Pheidole* species. We hypothesize that  
104 major mandibles are better able to mitigate stress than minors', given their greater robustness.  
105 Alternatively, if each worker type has mandibles optimized to perform different tasks, majors  
106 and minors could perform better in distinct biting scenarios. Differences between species are  
107 expected between the more distinct mandibles of majors, which can suggest changes in the  
108 capacity to deal with hard food items, given the specialized roles of those workers (Wilson  
109 1984; 2003). Alternatively, differences between species in minor worker mandibles will  
110 suggest that even small morphological distinctions can lead to biomechanical idiosyncrasies.

111 **Methods**

112 *Studied species*

113 Colonies of *Pheidole hetschkoi* Emery and *P. cf. lucretii* were collected in an urban  
114 fragment of Atlantic Forest in Curitiba, Paraná, Brazil (25°26'45.9"S 049°13'55.5"W).  
115 *Pheidole hetschkoi* is commonly found nesting fallen twigs; foraging in the species is based  
116 on the recruitment of tens of workers to food sources (rarely majors), and colonies  
117 accumulate seeds inside their nests (author's pers. obs.). On the other hand, *P. cf. lucretii*  
118 nests directly on the ground and might recruit up to hundreds of workers to food sources,  
119 including dozens of majors, but were never recorded collecting or consuming seeds (author's  
120 pers. obs). Morphologically, majors of *P. hetschkoi* are more sturdy, with larger heads  
121 (Fig.1b), with more robust mandibles than *P. cf. lucretii* majors, which have also smaller  
122 heads and are more slender in general (Fig.1a). Minors differ little between species in terms  
123 of mandible shape and general morphology (Fig. 1c and d). Voucher specimens are deposited  
124 at the Entomological Collection Padre Jesus Santiago Moure, Department of Zoology,  
125 Federal University of Paraná, Brazil.



126  
127 Fig. 1. *Pheidole* workers whole body 3D models in lateral view. (a) *P. cf. lucretii* major; (b)  
128 *P. hetschkoi* major; (c) *P. cf. lucretii* minor; (d) *P. hetschkoi* minor.

129

130 *CT scanning and image processing*

131 Workers scans were generated in a ZEISS Xradia 510 Versa X-ray microCT facility,  
132 using the software ZEISS Scout and Scan Control System, in the Biodiversity &  
133 Biocomplexity Unit, Okinawa Institute of Science and Technology Graduate University  
134 (OIST), Japan. Exposure time of each specimen varied from one to five seconds, under an  
135 “Air” filter and 4x objective. The voltage was set between 30 and 50keV, from 4 to 5W of  
136 power, under a “normal” field mode and intensity levels of 15,000 and 17,000 across the  
137 whole specimen. Scan time varied from 27 to 30 minutes, generating 801 projections from  
138 full 360-degree rotations. Model reconstruction was done on XMReconstructor, and  
139 mandibles segmentation was carried in ITK-snap 3.8.0 (Yushkevich et al., 2006). For mesh  
140 generation and simplification, we used the software MeshLab (Cignoni et al., 2008), and to  
141 generate the 3D mandible solid for FEA simulations we used the software Fusion 360  
142 (AUTODESK). Mandibles 3D solid models are available on the supplementary material.

143

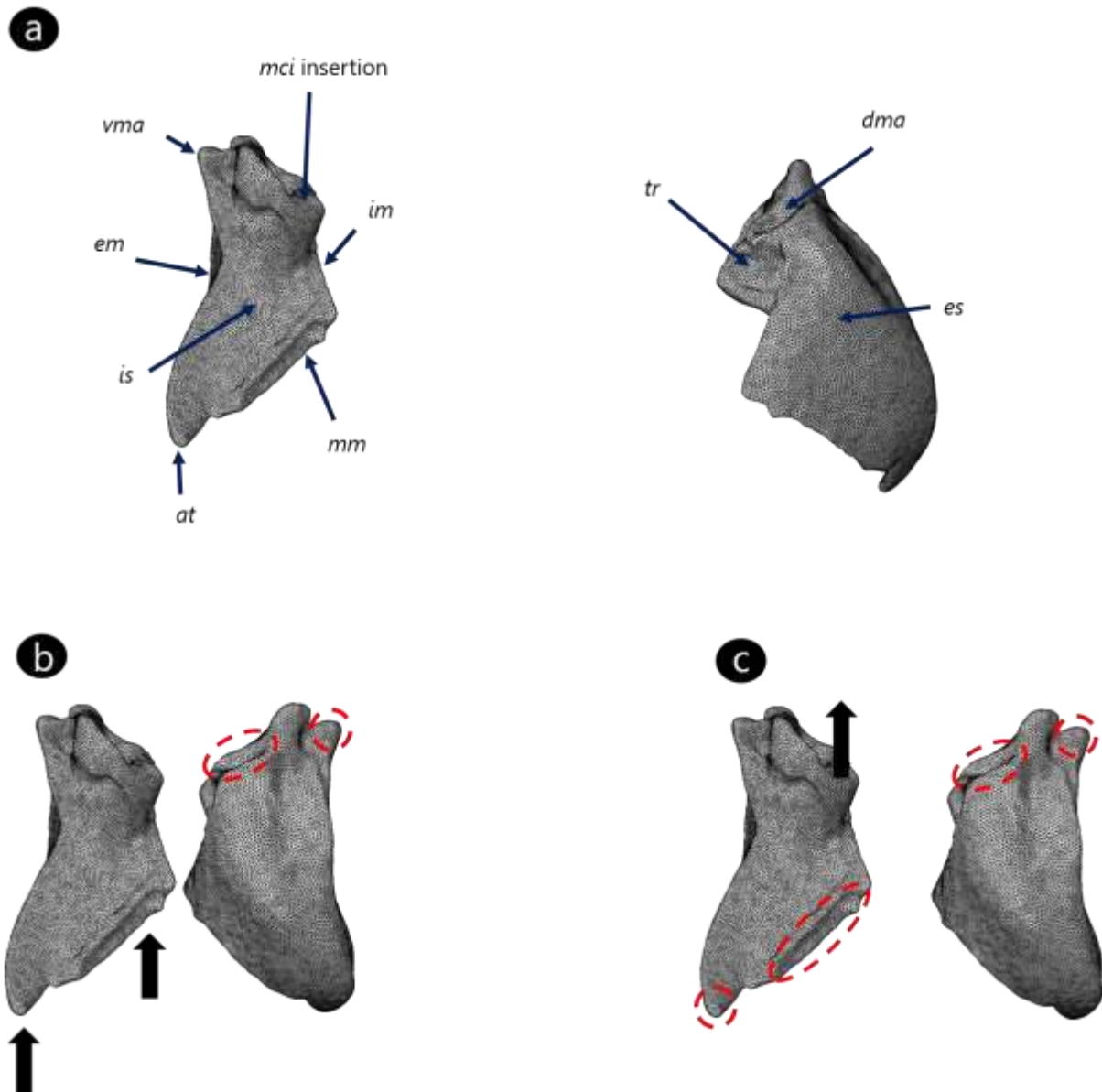
144 *FEA simulations*

145 To quantify the mechanical response of a structure to external loading, FEA requires  
146 the discretization of the structure into small parts, resulting in the finite element mesh  
147 composed of elements of pre-defined shape and a specific number of points, called nodes,  
148 used to solve the equations (Azevedo 2003, Marcé-Nogué et al., 2015). Displacements on  
149 nodes are calculated to estimate stress and strain, based on the structure material properties  
150 and shape (Rayfield 2007). We used 10-node tetrahedral elements (C3D10) to generate the  
151 finite element mesh. The number of elements varied for each model, as well as the size of  
152 each element between subcastes, to adapt meshes to each morphology (Table S11).

153 We performed linear static simulations of four distinct biting scenarios for each  
154 species and subcastes, divided into two categories, namely strike and pressure, which reflect  
155 different aspects of mandible movement in terms of force and velocity. In strike scenarios, a  
156 condition associated with faster movements, we define the mandible articulations with the  
157 head (dorsal - *dma* and ventral - *vma*) as the constrained regions, applying static load on the  
158 apical tooth or the masticatory margin (Figure 2a, b). In pressure scenarios, associated with  
159 slower mandible movements but powerful bites, in addition to the mandibular joints, we also

160 constrained the apical tooth or the masticatory margin and applied the load to the region of  
161 *mci* insertion, following the direction of contraction (Figure 2a, c) to simulate the use of  
162 mandibles for food compression. We constrained nodal displacement in x, y, and z directions  
163 and apply a 1 N load uniformly distributed among nodes in all simulations. We modeled the  
164 mandible cuticle as an isotropic and linearly elastic material, setting Young's modulus as  
165 2.75GPa and the Poisson's ratio as 0.3, based on measures from the cuticle of ant mandibles  
166 available in the literature (Brito et al., 2017). The only source of variation for each biting  
167 simulation between species and workers was the morphology of the mandibles. We present  
168 FEA stress results based on Tresca failure criterion, more suitable for brittle fracture, which  
169 determine an equivalent stress value under which the material will possibly fail when  
170 subjected to combined load (Özkaya et al., 2017). We used Abaqus 6 (Dassault Systèmes) to  
171 run the FEA simulations.





172

173 Fig. 2: Main mandibular regions considered to discuss stress dissipation patterns (a); Loaded  
174 and constrained regions in strike (b) and pressure (b) biting simulations. In b and c, arrows  
175 indicate the direction and region of load, and dashed lines enclose the constrained regions for  
176 each simulation. *at*: apical tooth; *dma*: dorsal mandibular articulation; *em*: external margin;  
177 *es*: external surface; *im*: internal margin; *is*: internal surface; *mci*: muscle craniomandibularis  
178 internus; *mm*: masticatory margin; *tr*: trulleum; *vma*: ventral mandibular articulation.

179           **Results**

180           *FEA simulations*

181           Stress distribution results are shown in Figure 3. Given that the volume of each  
182 model varies, and that we use idealized loads and material properties, we chose not to  
183 interpret absolute stress values. Rather, we will focus on qualitative differences among  
184 simulations by rescaling the stress ranges based on a reference model to facilitate  
185 comparisons between species, subcastes, and biting scenarios. Therefore, relative differences  
186 in stress distribution between simulations indicate mandibular biomechanical distinctions to  
187 assimilate loading conditions.

188

189           *Major worker mandibles*

190           When displacement restrictions were applied on the mandibular joints, those regions  
191 expectedly showed high-stress levels, but stresses had to spread to other regions to be  
192 effectively absorbed. Starting from the dorsal mandibular articulation (*dma*), stresses  
193 dissipate mainly along the mandible external surface and trulleum (Fig. 3c, d, g, h, k, l, o, p).  
194 Indeed, the trulleum was important to concentrate stresses coming from the *dma* in all  
195 simulations. Stresses from the ventral mandibular articulation (*vma*) spread mainly along the  
196 external margin and through its surroundings along internal and external surfaces (Fig. 3c, d,  
197 g, h, k, l, o, p). Contrasting different biting scenarios, higher stresses are found when only  
198 the apical tooth is employed (Fig. 3c, d, k, l). This result indicates that ants face marked  
199 mechanical restrictions when they only use the apical tooth. Pressure scenarios generated  
200 higher stresses around the basal region of the internal surface (Fig. 3k, l, o, p), whereas strike  
201 scenarios concentrated more stress near the masticatory margin, an expected consequence of  
202 load application (Fig. 3c, d, g, h). However, the key aspect relating different biting scenarios  
203 are the higher stress levels in *dma* and *vma* in the strike (Fig. 3c, d, g, h) versus pressure  
204 simulations (Fig. 3k, l, o, p), which indicates that strike causes higher mechanical demands  
205 in the mandibular joints than pressure.

206           The main aspect that influences stress dissipation differences between species is the  
207 mandible internal surface concavity. *Pheidole hetschkoi* has a deeper concavity near the  
208 masticatory margin, which acts as an important stress concentrator, mainly in strike scenarios  
209 on the masticatory margin (Fig. 2g). While *P. cf. lucretii* also shows stress concentration at

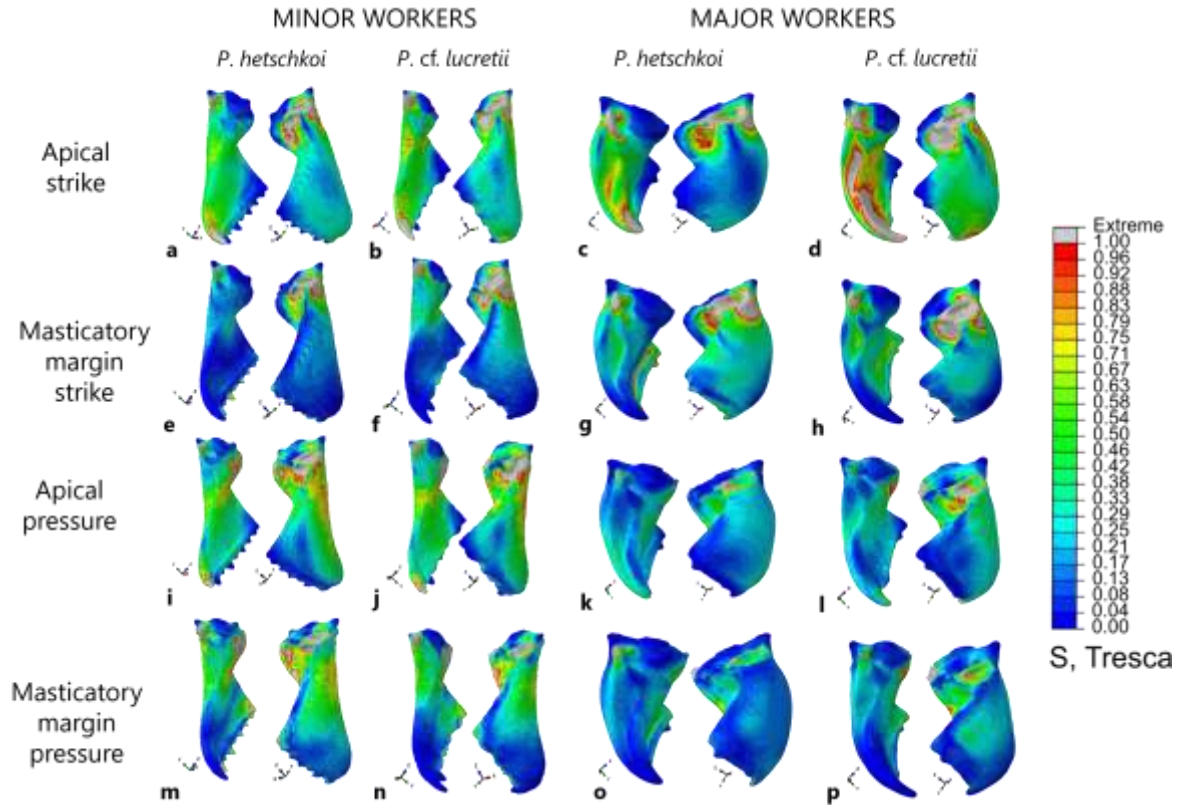
210 the same region in this biting scenario, those stresses spread more extensively along the  
211 internal surface (Fig. 3h), which suggests that its concavity is shallow and does not act as a  
212 stress concentrator. The external surface curvature also differs between species, but there are  
213 no substantial differences in terms of stress dissipation pattern (Fig. 3c, d, g, h, k, l, o, p). The  
214 dissipation through the external surface is more restricted to the articulations surroundings,  
215 given the robustness of the mandibular base, which could explain why there is not a  
216 conspicuous effect of the external surface curvature in the stress dissipation pattern between  
217 species. Stresses were proportionally higher in the *P. cf. lucretii* mandible, through most  
218 mandibular regions and all biting scenarios, but the differences are more striking in pressure  
219 scenarios (Fig. 3l, p).

220

#### 221 *Minor worker mandibles*

222 There is a distinguished stress concentration around the more constricted region of  
223 the mandibular internal surface, a trend that occurs mainly in strike simulations, especially  
224 when the load was applied on the masticatory margin (Fig. 3e, f). This constriction acts as a  
225 stress concentrator in minors due to their slender mandibles in comparison to majors. When  
226 the results of different species are compared, *P. cf. lucretii* simulations show proportionately  
227 higher stresses than *P. hetschkoi* in general (Fig. 3b, f, j, n), contrary to the expectation that  
228 minors mandibles would not differ in mechanical performance. The overall lower stress  
229 levels found in masticatory margin strike simulations of *P. hetschkoi* minors seems to reflect  
230 the presence of well-developed teeth along its masticatory margin. It is noticeable that the  
231 masticatory margin teeth absorb great levels of stress (Fig. 3e), so that their absence leads to  
232 higher stress levels along the mandible surfaces in strike simulations of *P. cf. lucretii* minor,  
233 as well as in majors of both species. The higher stresses along the internal surface in *P. cf.*  
234 *lucretii* minor mandible, compared to *P. hetschkoi* minor mandible, draw attention to the  
235 mechanical limitations associated with worn mandibles, as is the case of the *P. cf. lucretii*  
236 minor mandible modeled, which can lead to behavioral switches in task performance along  
237 the worker lifetime. Regarding the biting scenarios, pressure in minors result in higher  
238 stresses on internal and external surfaces of both species when compared to majors (Fig. 3i,  
239 j, m, n). As occurred in pressure scenarios for majors, stresses along the internal surface  
240 concentrate near the mandible base, where the load was applied. However, in minors, the

241 mandible base is slender, which can explain why the mandibular surfaces in minors are  
242 proportionally more stressed in pressure than in strike simulations.



243

244 Fig. 3: Tresca stress results (rescaled to range between 0-1) for the four biting scenarios  
245 (rows), from minors and majors of both *Pheidole* species (columns). Each letter depicts a  
246 distinct simulation. Color represents a proportional value of stress in relation to the maximum  
247 value considered for each simulation, indicated as 1.00, and grey represents extremes values  
248 above the maximum considered.

249           **Discussion**

250           The use of biomechanical simulations to explore the relationships between form and  
251 function in insects is a recent endeavor. To date, FEA simulations have been used to  
252 understand biomechanical consequences of male stag beetle mandibles during agonistic  
253 interactions (Goyens et al., 2014; 2015; 2016), as well as the morphological evolution of  
254 Anisoptera (Odonata) mandibles (Blanke et al., 2017). More recently, it was demonstrated  
255 how mandibles of the trap-jaw ant *Myrmica camillae* are morphologically adapted to deal  
256 with the loadings arising from the power amplification mechanism of its closing movement  
257 (Larabee et al., 2018). Here we apply FEA in mandibles of *Pheidole* workers to simulate  
258 different biting scenarios and investigate how morphological differences in mandible  
259 morphology reflects their responses to those bite loading demands.

260           Our results demonstrate how mandible morphology of dimorphic workers can be  
261 optimized for particular tasks and draws attention to the role of specific mandibular regions  
262 or structures to deal with the stresses generated by their bite. Ant workers have a typical  
263 pterygote triangular mandible (Snodgrass, 1935), which can be divided into two components,  
264 a basal thick stem, and a distal blade (Richter et al., 2020). Our results indicate that this  
265 distribution of cuticular material in the mandible may conform to the high loading demands  
266 experienced by the mandibular articulations with the head. Most of the stresses generated on  
267 the apical tooth dissipate along the external margin towards the mandibular base, in both  
268 species and subcastes, avoiding the spread of considerable stresses through the more delicate  
269 mandibular surfaces. In masticatory margin stress simulations, the presence of well-  
270 developed teeth results in stresses being concentrated on the teeth instead of spreading  
271 through the internal surface. Majors of *Pheidole*, in which the masticatory margin is  
272 toothless, show a higher degree of concavity on their internal surfaces, especially in *P.*  
273 *hetschkoi*, which helps to concentrate stresses near the more robust masticatory margin  
274 instead of spreading through the internal surface. Although alleviating the level of stress in  
275 the mandibular articulations, such stress concentration can be harmful in cases in which the  
276 structure is submitted to cycles of loading, leading to structural fail due to material fatigue  
277 (Dirks et al., 2013).

278           An important aspect of *Pheidole* mandibular morphology with respect to bite  
279 mechanics is the role of the trulleum on stress concentration. The trulleum is a concavity near

280 the *dma* present only in some myrmicine ants (Richter et al., 2019). The function of the  
281 trulleum was hitherto unknown. Here we demonstrate for the first time the importance of the  
282 trulleum to concentrate stresses coming from the *dma*, avoiding the spread of stresses through  
283 the more delicate mandibular surfaces. This is an interesting discovery, given that the *dma*  
284 seems to concentrate higher stresses in general than the *vma*. Given the trulleum clear  
285 functional role here outlined, it would be interesting to investigate the biomechanical  
286 responses of mandibles that lack the development of the trulleum, to understand how stresses  
287 dissipate from *dma* without this important stress concentrator, especially in ant species with  
288 similar loading demands as *Pheidole* mandibles.

289         Our results also underscore how the more robust major mandibles are better suited  
290 to deal with pressure biting than minors slender mandibles, which show higher performance  
291 in strike scenarios. As expected, these results agree with the specialized roles played by major  
292 workers in the colony. The behavioral repertoire of major workers is particularly limited,  
293 being frequently restricted to defense and/or food processing (Wilson 1984; 2003). Indeed,  
294 when minors are experimentally removed from the colony, major workers take over many of  
295 their tasks, although with decreased efficiency (Wilson 1984; Mertl & Traniello, 2009).  
296 Major mandibles meet the demands to deal with the processing of hard food items through  
297 pressure, with their toothless masticatory margin spreading bite forces evenly around the  
298 food item. Seed consumption is considered an important aspect in the evolution of several  
299 myrmicine genera, such as *Pheidole*, *Pogonomyrmex*, and *Solenopsis* (Ferster et al., 2006;  
300 Moreau 2008). However, the influence of granivory on morphological evolution, especially  
301 regarding the dimorphism in the *Pheidole* worker caste, is still poorly known (Holley et al.,  
302 2016). Here, we demonstrate for the first time how ant mandible morphology can be tuned  
303 to deal with the mechanical demands of processing hard food items, such as seeds and  
304 arthropod cuticles, through major mandibles better performance in pressure biting conditions.  
305 Also, mandibles of *P. hetschkoi* majors show an even better performance in pressure bite  
306 than *P. cf. lucretii*, suggesting that majors of *P. hetschkoi* can deal better with harder food  
307 items than *P. cf. lucretii*. These results may lead to the possibility of food partitioning among  
308 *Pheidole* coexisting species, and agrees with the habit of seed consumption by *P. hetschkoi*,  
309 which demands higher bite forces and consequently leads to higher stress levels on the  
310 mandibles.



311           Although minor workers of *Pheidole* typically show well-developed teeth in their  
312 masticatory margin, the particular specimen of *P. cf. lucretii* included in our study showed  
313 high levels of teeth wear, allowing us to assess the consequences of teeth wear on bite  
314 loadings. Teeth concentrate the forces generated by the masticatory muscles on smaller areas,  
315 improving the initiation of the fracture (Clissold 2007). The importance of teeth to task  
316 efficiency was demonstrated for leaf-cutting ants, where workers specialized to cut leaves  
317 switch to carrying them once their teeth are worn to a certain degree, reducing their cutting  
318 efficiency (Schofield et al., 2011). In *Pheidole*, minors perform a wide range of tasks in the  
319 colony (Wilson 1984; 2003), but information on the role of teeth wear in minor task switching  
320 is scarce. Here we demonstrate the possible mechanical consequences of teeth wear in ant  
321 mandibles, comparing the relative amount of stress generated during masticatory margin  
322 strike simulations in *P. hetschkoi* and *P. cf. lucretii* minors. Our results indicated that *P. cf.*  
323 *lucretii* has relatively higher stresses than *P. hetschkoi*, mainly along its mandible internal  
324 surface, which drives to higher stresses at the mandibular articulations with the head. Further  
325 studies in task allocation and mandible morphology in dimorphic ants colonies can address  
326 if teeth wear generates task switch, and biomechanical studies can reveal how teeth wear  
327 reduces task efficiency (Schofield et al., 2011).

328           The morphological evolution of *Pheidole* might be strongly driven by differences in  
329 size (Pie & Traniello, 2007), which tends to evolve at higher rates than shape (Pie & Tschá,  
330 2013; Economo et al., 2015a; Friedman et al., 2019). More recently, studies applying  
331 geometric morphometric approaches validated the prominence of size to explain the  
332 morphological disparity in the genus but also pointed to different evolutionary rates and  
333 levels of integration between head and mesosoma shape and size (Friedman et al., 2019;  
334 2020). *Pheidole* morphological diversification seems to be very constrained (Pie & Traniello,  
335 2007), in contrast to their ecological disparity (Economo et al., 2015a; 2015b), as reflected  
336 in the widespread distribution of genus throughout most of the terrestrial ecosystems  
337 (Economo et al., 2019). Field observations demonstrate that, despite the relative  
338 morphological resemblance in *Pheidole* species, they can show considerable ecological and  
339 behavioral diversity (Mertl et al., 2010; Tschá & Pie, 2019). We demonstrate that even small  
340 morphological differences in mandibles shape between species can lead to biomechanical  
341 specialization, mainly in terms of the food processing capacity of majors. This biomechanical



342 specialization can expand the diet range of species and lead to food partitioning (Blüthgen et  
343 al., 2003; Rosumek 2017), decreasing the degree of competition and allowing for species  
344 coexistence (Blüthgen & Feeldar, 2010).

345 Our results provide a biomechanical basis to understand how mandible  
346 morphological evolution can improve task specialization in polymorphic ants. Morphological  
347 polymorphism in the worker caste can expand the range of prey items that a species is able  
348 to handle, as demonstrated for some species of the army ant genus *Eciton* (Powell & Franks,  
349 2005; 2006). In the highly polymorphic genus *Cephalotes*, which is *Pheidole*'s sister lineage,  
350 some workers are specialized to plug the nest entrances with their heads to protect the colony  
351 against invasion (Powell 2008). In some fire ants such as *Solenopsis geminate* (Fabricius),  
352 the degree of worker polymorphism is associated to higher levels of division of labor, with  
353 major workers being specialized to seed milling (Wilson 1978; Ferster et al., 2006). Division  
354 of labor in leaf-cutting ants is associated with morphological distinctions among worker  
355 mandibles, as demonstrated for the polymorphic genus *Atta* (Silva et al., 2016). Therefore,  
356 the role of worker polymorphism for division of labor in ants is well established (Wills et al.,  
357 2018), but by applying biomechanical approaches we can advance our understanding about  
358 the functional role of morphological disparity, as we demonstrated here for *Pheidole* workers.  
359 In this sense, ant polymorphic lineages are ideal models to investigate form-function  
360 relationships, and the morphological differentiation of mandibles should be studied in detail,  
361 given the importance of this structure to worker interactions with the environment.

362

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### 369 **References**

370 Azevedo AF. 2003 *Método dos Elementos Finitos*. Porto: Faculdade de Engenharia da  
371 Universidade do Porto.

- 372 Blanke A, Schmitz H, Patera A, Dutel H, Fagan MJ. 2017 Form–function relationships in  
373 dragonfly mandibles under an evolutionary perspective. *J. R. Soc. Interface* 14, 20161038.  
374 (doi:10.1098/rsif.2016.1038)
- 375 Blüthgen N, Feldhaar H. 2010 Food and shelter: how resources influence ant ecology. In *Ant*  
376 *Ecology*, pp. 115–136. New York: Oxford University Press.
- 377 Blüthgen N, Gebauer G, Fiedler K. 2003 Disentangling a rainforest food web using stable  
378 isotopes: dietary diversity in a species-rich ant community. *Oecologia* 137, 426–435.  
379 (doi:10.1007/s00442-003-1347-8)
- 380 Bolton B. An online catalog of the ants of the world. 2020. antcat.org (last access in  
381 03/SEP/2020).
- 382 Brito TO, Elzubair A, Araújo LS, Camargo SA de S, Souza JLP, Almeida LH. 2017  
383 Characterization of the Mandible *Atta Laevigata* and the Bioinspiration for the Development  
384 of a Biomimetic Surgical Clamp. *Mat. Res.* 20, 1525–1533. (doi:10.1590/1980-5373-mr-  
385 2016-1137)
- 386 Cignoni P, Callieri M, Corsini M, Dellepiane M, Ganovelli F, Ranzuglia G. 2008 Meshlab:  
387 an open-source mesh processing tool. In *Eurographics Italian chapter conference*, pp. 129–  
388 136.
- 389 Clissold FJ. 2007 The biomechanics of chewing and plant fracture: mechanisms and  
390 implications. *Adv In Insect Phys* 34, 317–372.
- 391 Dirks J-H, Parle E, Taylor D. 2013 Fatigue of insect cuticle. *Journal of Experimental Biology*  
392 216, 1924–1927. (doi:10.1242/jeb.083824)
- 393 Economo EP, Huang J-P, Fischer G, Sarnat EM, Narula N, Janda M, Guénard B, Longino  
394 JT, Knowles LL. 2019 Evolution of the latitudinal diversity gradient in the hyperdiverse ant  
395 genus *Pheidole*. *Global Ecol Biogeogr* 28, 456–470. (doi:10.1111/geb.12867)
- 396 Economo EP, Klimov P, Sarnat EM, Guénard B, Weiser MD, Lecroq B, Knowles LL. 2015a  
397 Global phylogenetic structure of the hyperdiverse ant genus *Pheidole* reveals the repeated  
398 evolution of macroecological patterns. *Proc. R. Soc. B* 282, 20141416.  
399 (doi:10.1098/rspb.2014.1416)

- 400 Economo EP *et al.* 2015b Breaking out of biogeographical modules: range expansion and  
401 taxon cycles in the hyperdiverse ant genus *Pheidole*. *J. Biogeogr.* 42, 2289–2301.  
402 (doi:10.1111/jbi.12592)
- 403 Evans AR, Sanson GD. 2005 Biomechanical properties of insects in relation to insectivory:  
404 cuticle thickness as an indicator of insect ‘hardness’ and ‘intractability’. *Aust. J. Zool.* 53, 9.  
405 (doi:10.1071/ZO04018)
- 406 Ferster B, Pie MR, Traniello JFA. 2006 Morphometric variation in North American  
407 Pogonomyrmex and Solenopsis ants: caste evolution through ecological release or dietary  
408 change? *Ethology Ecology & Evolution* 18, 19–32. (doi:10.1080/08927014.2006.9522723)
- 409 Friedman NR, Lecroq Bennet B, Fischer G, Sarnat EM, Huang J, Knowles LLK, Economo  
410 EP. 2020 Macroevolutionary integration of phenotypes within and across ant worker castes.  
411 *Ecol Evol*, ece3.6623. (doi:10.1002/ece3.6623)
- 412 Friedman NR, Remeš V, Economo EP. 2019 A morphological integration perspective on the  
413 evolution of dimorphism among sexes and social insect castes. *Integr Comp Biol* 59, 410–  
414 419.
- 415 Gadagkar R. 1997 The evolution of caste polymorphism in social insects: Genetic release  
416 followed by diversifying evolution. *J. Genet.* 76, 167–179. (doi:10.1007/BF02932215)
- 417 Goyens J, Dirckx J, Aerts P. 2015 Built to fight: variable loading conditions and stress  
418 distribution in stag beetle jaws. *Bioinspir. Biomim.* 10, 046006. (doi:10.1088/1748-  
419 3190/10/4/046006)
- 420 Goyens J, Dirckx J, Aerts P. 2016 Jaw morphology and fighting forces in stag beetles. *J Exp*  
421 *Biol* 219, 2955–2961. (doi:10.1242/jeb.141614)
- 422 Goyens J, Soons J, Aerts P, Dirckx J. 2014 Finite-element modelling reveals force  
423 modulation of jaw adductors in stag beetles. *J. R. Soc. Interface* 11, 20140908.  
424 (doi:10.1098/rsif.2014.0908)
- 425 Gronenberg W, Paul J, Just S, Hölldobler B. 1997 Mandible muscle fibers in ants: fast or  
426 powerful? *Cell and Tissue Research* 289, 347–361. (doi:10.1007/s004410050882)

- 427 Hao W, Yao G, Zhang X, Zhang D. 2018 Kinematics and Mechanics analysis of trap-jaw ant  
428 *Odontomachus monticola*. *J. Phys.: Conf. Ser.* 986, 012029. (doi:10.1088/1742-  
429 6596/986/1/012029)
- 430 Hölldobler B, Wilson EO. 1990 *The ants*. Cambridge, Mass: Belknap Press of Harvard  
431 University Press.
- 432 Holley J-AC, Moreau CS, Laird JG, Suarez AV. 2016 Subcaste-specific evolution of head  
433 size in the ant genus *Pheidole*. *Biol. J. Linn. Soc.* 118, 472–485. (doi:10.1111/bij.12769)
- 434 Huang MH, Wheeler DE, Fjerdingstad EJ. 2013 Mating system evolution and worker caste  
435 diversity in *Pheidole* ants. *Mol Ecol* 22, 1998–2010. (doi:10.1111/mec.12218)
- 436 Larabee FJ, Smith AA, Suarez AV. 2018 Snap-jaw morphology is specialized for high-speed  
437 power amplification in the Dracula ant, *Mystridium camillae*. *R. Soc. open sci.* 5, 181447.  
438 (doi:10.1098/rsos.181447)
- 439 Lillico-Ouachour A, Metscher B, Kaji T, Abouheif E. 2018 Internal head morphology of  
440 minor workers and soldiers in the hyperdiverse ant genus *Pheidole*. *Can. J. Zool.* 96, 383–  
441 392. (doi:10.1139/cjz-2017-0209)
- 442 Marcé-Nogué J, Fortuny J, Gil L, Sánchez M. 2015 Improving mesh generation in finite  
443 element analysis for functional morphology approaches. *Spanish J. Palaeontol.* 30, 117-132.  
444 (doi:10.7203/sjp.30.1.17227)
- 445 Mertl AL, Sorenson MD, Traniello JFA. 2010 Community-level interactions and functional  
446 ecology of major workers in the hyperdiverse ground-foraging *Pheidole* (Hymenoptera,  
447 Formicidae) of Amazonian Ecuador. *Insect. Soc.* 57, 441–452. (doi:10.1007/s00040-010-  
448 0102-5)
- 449 Mertl AL, Traniello JFA. 2009 Behavioral evolution in the major worker subcaste of twig-  
450 nesting *Pheidole* (Hymenoptera: Formicidae): does morphological specialization influence  
451 task plasticity? *Behav Ecol Sociobiol* 63, 1411–1426. (doi:10.1007/s00265-009-0797-3)
- 452 Moreau CS. 2008 Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole*  
453 (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution* 48, 224–239.  
454 (doi:10.1016/j.ympev.2008.02.020)

- 455 Muscedere ML, Traniello JFA, Gronenberg W. 2011 Coming of age in an ant colony:  
456 cephalic muscle maturation accompanies behavioral development in *Pheidole dentata*.  
457 *Naturwissenschaften* 98, 783–793. (doi:10.1007/s00114-011-0828-6)
- 458 Neville AC. 1975 *General Structure of Integument*. In *Biology of the Arthropod Cuticle*, pp.  
459 7–70. Berlin: Springer -Verlag.
- 460 Oliver WC, Pharr GM. 1992 An improved technique for determining hardness and elastic  
461 modulus using load and displacement sensing indentation experiments. *J. Mater. Res.* 7,  
462 1564–1583. (doi:10.1557/JMR.1992.1564)
- 463 Oster GF, Wilson EO 1978 *Caste and Ecology in the Social Insects*. New Jersey: Princeton  
464 University Press.
- 465 Özkaya N, Leger D, Goldsheyder D, Nordin M. 2017 Multiaxial Deformations and Stress  
466 Analyses. In *Fundamentals of Biomechanics: Equilibrium, Motion, and Deformation*, pp.  
467 317–360. New York: Springer.
- 468 Paul J. 2001 Mandible movements in ants. *Comparative Biochemistry and Physiology Part*  
469 *A: Molecular & Integrative Physiology* 131, 7–20. (doi:10.1016/S1095-6433(01)00458-5)
- 470 Paul J, Gronenberg W. 1999 Optimizing force and velocity: mandible muscle fibre  
471 attachments in ants. *Journal of Experimental Biology* 202, 797–808.
- 472 Pie MR, Traniello JFA. 2007 Morphological evolution in a hyperdiverse clade: the ant genus  
473 *Pheidole*. *J Zoology* 271, 99–109. (doi:10.1111/j.1469-7998.2006.00239.x)
- 474 Pie MR, Tschá MK. 2013 Size and shape in the evolution of ant worker morphology. *PeerJ*  
475 1, e205. (doi:10.7717/peerj.205)
- 476 Powell S, Franks NR. 2005 Caste evolution and ecology: a special worker for novel prey.  
477 *Proc. R. Soc. B* 272, 2173–2180. (doi:10.1098/rspb.2005.3196)
- 478 Powell S. 2008 Ecological specialization and the evolution of a specialized caste in  
479 *Cephalotes* ants. *Functional Ecology* 22, 902–911. (doi:10.1111/j.1365-2435.2008.01436.x)
- 480 Powell S, Franks NR. 2006 Ecology and the evolution of worker morphological diversity: a  
481 comparative analysis with *Eciton* army ants. *Funct Ecology* 20, 1105–1114.  
482 (doi:10.1111/j.1365-2435.2006.01184.x)

- 483 Powell S. 2009 How ecology shapes caste evolution: linking resource use, morphology,  
484 performance and fitness in a superorganism. *Journal of Evolutionary Biology* 22, 1004–1013.  
485 (doi:10.1111/j.1420-9101.2009.01710.x)
- 486 Rajabi H, Ghoroubi N, Darvizeh A, Dirks J-H, Appel E, Gorb SN. 2015 A comparative study  
487 of the effects of vein-joints on the mechanical behaviour of insect wings: I. Single joints.  
488 *Bioinspir. Biomim.* 10, 056003. (doi:10.1088/1748-3190/10/5/056003)
- 489 Rajabi H, Ghoroubi N, Darvizeh A, Appel E, Gorb SN. 2016 Effects of multiple vein  
490 microjoints on the mechanical behaviour of dragonfly wings: numerical modelling. *R. Soc.*  
491 *open sci.* 3, 150610. (doi:10.1098/rsos.150610)
- 492 Rayfield EJ. 2007 Finite Element Analysis and Understanding the Biomechanics and  
493 Evolution of Living and Fossil Organisms. *Annu. Rev. Earth Planet. Sci.* 35, 541–576.  
494 (doi:10.1146/annurev.earth.35.031306.140104)
- 495 Richter A, Hita Garcia F, Keller RA, Billen J, Economo EP, Beutel RG. 2020 Comparative  
496 analysis of worker head anatomy of *Formica* and *Brachyponera* (Hymenoptera: Formicidae).  
497 *Arthropod Systematics & Phylogeny* 78, 133–170. (doi:10.26049/ASP78-1-2020-06)
- 498 Richter A, Keller RA, Rosumek FB, Economo EP, Hita Garcia F, Beutel RG. 2019 The  
499 cephalic anatomy of workers of the ant species *Wasmannia affinis* (Formicidae,  
500 Hymenoptera, Insecta) and its evolutionary implications. *Arthropod Structure &*  
501 *Development* 49, 26–49. (doi:10.1016/j.asd.2019.02.002)
- 502 Rosumek FB. 2017 Natural History of Ants: What We (do not) Know About Trophic and  
503 Temporal Niches of Neotropical Species. *Sociobiology* 64, 244.  
504 (doi:10.13102/sociobiology.v64i3.1623)
- 505 Sarnat EM, Friedman NR, Fischer G, Lecroq-Bennet B, Economo EP. 2017 Rise of the spiny  
506 ants: diversification, ecology and function of extreme traits in the hyperdiverse genus  
507 *Pheidole* (Hymenoptera: Formicidae). *Biological Journal of the Linnean Society* 122, 514–  
508 538. (doi:10.1093/biolinnean/blx081)
- 509 Schofield RMS, Emmett KD, Niedbala JC, Nesson MH. 2011 Leaf-cutter ants with worn  
510 mandibles cut half as fast, spend twice the energy, and tend to carry instead of cut. *Behav*  
511 *Ecol Sociobiol* 65, 969–982. (doi:10.1007/s00265-010-1098-6)

- 512 Silva LC, Camargo RS, Lopes JFS, Forti LC. 2016 Mandibles of Leaf-Cutting Ants:  
513 Morphology Related to Food Preference. *Sociobiology* 63, 881.  
514 (doi:10.13102/sociobiology.v63i3.1014)
- 515 Sirohi J, Chopra I. 2000 Fundamental Understanding of Piezoelectric Strain Sensors. *Journal*  
516 *of Intelligent Material Systems and Structures* 11, 246–257. (doi:10.1106/8BFB-GC8P-  
517 XQ47-YCQ0)
- 518 Snodgrass RE, 1935 *Principles of Insect Morphology*. New York: Cornell University Press.
- 519 Sun J, Tong J, Ma Y. 2008 Nanomechanical Behaviours of Cuticle of Three Kinds of Beetle.  
520 *Journal of Bionic Engineering* 5, 152–157. (doi:10.1016/S1672-6529(08)60087-6)
- 521 Tschá MK, Pie MR. 2019 Correlates of ecological dominance within *Pheidole* ants  
522 (Hymenoptera: Formicidae): Correlates of ecological dominance in ants. *Ecol Entomol* 44,  
523 163–171. (doi:10.1111/een.12685)
- 524 Vincent JFV, Wegst UGK. 2004 Design and mechanical properties of insect cuticle.  
525 *Arthropod Structure & Development* 33, 187–199. (doi:10.1016/j.asd.2004.05.006)
- 526 Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, Gerig G. 2006 User-guided  
527 3D active contour segmentation of anatomical structures: Significantly improved efficiency  
528 and reliability. *NeuroImage* 31, 1116–1128. (doi:10.1016/j.neuroimage.2006.01.015)
- 529 Wheeler DE. 1991 The Developmental Basis of Worker Caste Polymorphism in Ants. The  
530 *American Naturalist* 138, 1218–1238. (doi:10.1086/285279)
- 531 Wheeler WM. 1910. *Ants: their structure, development and behavior*. New York: Columbia  
532 University Press.
- 533 Wills BD, Powell S, Rivera MD, Suarez AV. 2018 Correlates and Consequences of Worker  
534 Polymorphism in Ants. *Annu. Rev. Entomol.* 63, 575–598. (doi:10.1146/annurev-ento-  
535 020117-043357)
- 536 Wilson EO. 1978 Division of labor in fire ants based on physical castes (Hymenoptera:  
537 Formicidae: *Solenopsis*). *Journal of the Kansas Entomological Society* 51, 615–636.
- 538 Wilson EO. 2003 *Pheidole* in the New World: a dominant, hyperdiverse ant genus.  
539 Cambridge, Mass: Harvard University Press.



- 540 Wilson EO. 1971 *The insect societies*. Cambridge, EUA: Harvard University Press.
- 541 Wilson EO. 1953 The Origin and Evolution of Polymorphism in Ants. *The Quarterly Review*  
542 *of Biology* 28, 136–156. (doi:10.1086/399512)
- 543 Wilson EO. 1984 The relation between caste ratios and division of labor in the ant  
544 genus *Pheidole* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 16, 89–98.  
545 (doi:10.1007/BF00293108)
- 546



547 **Supporting Information**

548 Table SI1: Characteristics of each finite element mesh.

	Specimen	Mesh volume (mm <sup>3</sup> )	Element edge length (mm)	Number of elements
Major	<i>P. hetschkoi</i>	0.0741	0.023	449488
	<i>P. cf. lucretii</i>	0.0183	0.023	278634
Minor	<i>P. hetschkoi</i>	0.00469	0.0035	881691
	<i>P. cf. lucretii</i>	0.00191	0.0035	392790

549