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

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Statement (if applicable):

Three-dimensional reconstructions of the Pheidole workers mandibles used in the Finite Element Analysis simulations are available as Supplementary Material File S1 at ["figshare repository"](#).

Conflict of interest

I/We declare we have no competing interests

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Authors' contributions

This paper has multiple authors and our individual contributions were as below

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ACF and EPE collected the raw data; CLK, ACF, MAA and MRP contributed on the study design; CLK, ACF and MAA contributed on data analysis; CLK and MRP drafted the manuscript; ACF, EPE and MAA critically revised the manuscript; MRP coordinated the study.

31 Introduction

32 The evolution of complex societies in ants followed the advent of reproductive
33 division of labor into distinct castes, in which largely sterile and wingless individuals (i.e.,
34 workers) perform quotidian colony tasks, whereas winged individuals became specialized
35 for colony reproduction (i.e., queens and males) [1,2]. These changes were accompanied by
36 substantial morphological differences among reproductives and non-reproductives, with the
37 latter giving up reproduction and dispersal capacities while experiencing both morphological
38 and behavioral specialization [3-5]. In some ant lineages, the worker force became further
39 subdivided into morphologically distinct subcastes (e.g., minor vs. major workers), and such
40 differences are thought to allow differential performance of particular roles in the colony,
41 such as seed milling and defense [6,7]. In ants, worker polymorphism evolved in several
42 lineages, and its role in facilitating task specialization is widely recognized [6-8]. Several
43 studies explored the genetic [9,10], ecological [11-13], and developmental [14,15]
44 determinants of worker polymorphism in distinct ant lineages [8].

45 The genus *Pheidole* shows an interesting pattern among its almost 1,200 known
46 species [16]: the development of dimorphic worker subcastes, represented by major and
47 minor workers, where majors have a disproportionately larger head [6,15,17]. *Pheidole*
48 species are distributed worldwide, but most of their diversity and abundance is concentrated
49 in the tropics [18,19]. Although *Pheidole* species are typically considered diet generalists
50 [17], some species might show some degree of dietary specialization [20]. Of all their food
51 items, feeding on seeds evolved many independent times and has been indicated as an
52 important factor to explain the lineage diversification due to behavioral and morphological
53 adaptations related to seed harvesting and processing [21]. Since majors are specialized in
54 tasks such as defense and food processing [17,22], their larger heads could be a consequence
55 of evolutionary pressures towards the specialization to those tasks [23]. However, evidence
56 gathered so far has been mixed (e.g., [24]).

57 Understanding the main trends in the morphological evolution of *Pheidole* has
58 received considerable attention in the past decade. Different approaches were employed to
59 understand the evolution of a variety of structures, showing contrasting results to the relative
60 contributions of size and shape to the morphological diversity of the genus [23,25-28].
61 However, little is known about the evolution of mandibular morphology in *Pheidole*. The

1
2
3 62 proximal articulations of ant mandibles are dicondylic, as expected for Pterygota [29], with
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5 63 both dorsal and ventral joints [30]. Ant worker mandibles are the primary structures used to
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7 64 interact with their environment (e.g., biting, carrying, excavating, cutting, fighting) [31].
8
9 65 Mandibular movement is powered by two muscles, the *craniomandibularis internus* (Omd1),
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11 66 whose contraction closes the mandibles, and the *craniomandibularis externus* (Omd3),
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13 67 responsible for the opening process [29,30]. The Omd1 fibers attach to the mandible through
14
15 68 a mandibular cuticular projection called mandibular apodeme [32]. The angle of attachment
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17 69 to the apodeme, combined with sarcomere length, are directly related to the velocity and
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19 70 force of the mandibular movement [33], so that Omd1 is considered the key to the versatility
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21 71 of ant mandibles [34,35], being much more developed than the Omd3 [30,32,35]. In *Pheidole*
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23 72 majors the Omd1 is remarkably large, with its increase in size compared with minors being
24
25 73 achieved at the expense of the glandular, digestive, and nervous system in the head [36].
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27 74 Fibers of the Omd1 also continue to develop even for days after the adult emergence in both
28
29 75 subcastes, and this characteristic correlates to behavioral development in workers [37].

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31 76 Regardless of the importance of mandibles to many aspects of ant life history, little
32
33 77 is known about how morphological variation between species or worker subcastes relates to
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35 78 bite loading demands, except for one specialized snap-jaw species [38]. Worker
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37 79 polymorphism can lead to behavioral specialization, mainly through variation in mandible
38
39 80 morphology [39-41], but biomechanical approaches to directly assess this relationship in ants
40
41 81 are scarce [38]. To understand how mandible morphology relates to the biomechanical
42
43 82 demands of biting, it is important to employ approaches that allow for the direct assessment
44
45 83 of bite loading conditions. Finite Element Analysis (FEA) is a numerical method that
46
47 84 approximates the mechanical simulation of loading conditions in structures of interest. By
48
49 85 applying loads and defining the boundary conditions (movement restrictions) on the structure,
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51 86 FEA estimates the mechanical response, i.e., how stress flows along the structure according
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53 87 to its shape [42,43]. By employing FEA, one can assess how variation in mandibular
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55 88 morphology among ant species as well as between castes and subcastes translates into the
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57 89 capacity of mandibles to deal with bite loading demands [38], as also explored for the
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59 90 evolution of mandible form in dragonflies [44], stag beetles [45-47], and the functional
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91 morphology of the mouthparts of the reticulated beetle *Priacma serrata* (LeConte) [48].
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93 Biomechanical approaches employing FEA have also revealed important aspects of the

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2
3 93 evolution of other insect structures, such as wings and the mechanics of flight [49,50] and
4 94 the evolution of insect head capsules [51,52].

5
6 95 To improve our understanding of morphological evolution in *Pheidole* species, and
7 96 the role of morphological differentiation to improve task specialization in polymorphic ants,
8 97 we simulate several bite scenarios *in silico* by applying FEA [42,43] on 3D models of minor
9 98 and major mandibles of two *Pheidole* species. We hypothesize that major mandibles are
10 99 better able to mitigate stress than those of minors, given their greater robustness.
11 100 Alternatively, if each worker subcaste has mandibles optimized to perform different tasks,
12 101 majors and minors could perform better in distinct biting scenarios. Interspecific differences
13 102 are expected between the more distinct mandibles of majors, which can suggest changes in
14 103 the capacity to deal with hard food items, given the specialized roles of those workers [17,22].
15 104 Alternatively, differences between species in minor worker mandibles will suggest that even
16 105 small morphological distinctions can lead to biomechanical idiosyncrasies.
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106 **Methods**

107 *Studied species*

108 Colonies of *Pheidole hetschkoi* Emery and *P. cf. lucretii* were collected in an urban
109 fragment of Atlantic Forest in Curitiba, Paraná, Brazil (25°26'45.9"S 49°13'55.5"W). Those
110 species were selected due to their high abundance and ease of collection in the study area, as
111 well as because of the morphological discrepancies observed mainly between major worker
112 mandibles, which suggested possible biomechanical idiosyncrasies in the response to bite
113 loading demands that could affect species dietary amplitudes. Majors of *P. hetschkoi* are
114 sturdy, with larger heads (Fig.1a) and more robust mandibles than *P. cf. lucretii* majors,
115 which have also smaller heads and are slender (Fig.1c). Minors differ little between species
116 in mandible shape (Fig. 1b and d). *Pheidole hetschkoi* harvest and accumulate seeds in their
117 nests, which was never recorded for *P. cf. lucretii* (author's pers. obs.). Voucher specimens
118 are deposited at the Entomological Collection Padre Jesus Santiago Moure, Department of
119 Zoology, Federal University of Paraná, Brazil.

121 *CT scanning and image processing*

122 One specimen of each subcaste and species were scanned with a ZEISS Xradia 510
123 Versa X-ray microCT scanner at the Okinawa Institute of Science and Technology, using the
124 software ZEISS Scout and Scan Control System. Exposure time of each specimen varied
125 from one to five seconds, under an "Air" filter and 4x objective. The voltage was set between
126 30 and 50keV, from 4 to 5W of power, under a "normal" field mode and intensity levels of
127 15,000 and 17,000 across the whole specimen. Scan time varied from 27 to 30 minutes,
128 generating 801 projections from full 360-degree rotations. Model reconstruction was
129 performed with XMReconstructor, and mandibles segmentation was carried in ITK-snap
130 3.8.0 [53]. For mesh generation and simplification, we used the software MeshLab [54], and
131 to generate 3D mandible models for FEA simulations we used the software Fusion 360
132 (AUTODESK). Ant mandibles are internally hollow, and their cuticle varies in thickness
133 along the mandible axis, characteristics that can influence mechanical behavior and structure
134 stiffness, and we incorporated these aspects in our 3D reconstructions to model realistic
135 mandible morphologies (Supplementary Material File S1).

136

137 *FEA simulations*

138 FEA is a numerical method that approximates the mechanical responses of a
139 structure submitted to loading demands [43] which, in the case of biological structures, could
140 represent the demands of biting, running, jumping, and so on [42]. Here, the structures of
141 interest are the *Pheidole* worker mandibles, and the loading demands refer to different bite
142 conditions. To quantify the mechanical response of a structure to external loading, FEA
143 requires the discretization of the structure into small parts, resulting in the finite element
144 mesh composed of elements of pre-defined shape and a specific number of points, called
145 nodes, used to solve the equations [55,56]. Displacements on nodes are calculated to estimate
146 stress and strain based on the structure's material properties and shape [42]. We used 10-
147 node tetrahedral elements (C3D10) to generate the finite element mesh. The number of
148 elements varied for each model, as well as the size of each element between subcastes, to
149 adapt meshes to each morphology (Table 1).

150 We performed linear static simulations of four distinct biting scenarios for each
151 species and subcastes, divided into two categories, namely strike and pressure, which reflect
152 different aspects of mandible movement in terms of force and velocity. In all simulations, we
153 defined the constrained and loaded regions to capture the mechanical response at the exact
154 moment that the mandible hits or presses an object. Therefore, we did not intend to simulate
155 the conditions during the mandibular closing movement. In strike scenarios, a condition
156 associated with faster movements, we define the mandible articulations with the head (dorsal
157 - *dma* and ventral - *vma*) as the constrained regions, applying static load on the apical tooth
158 or the masticatory margin (*at* and *mm*, Figure 2a). 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 *mm*. We applied the load to the region of *0md1* insertion,
161 following the direction of contraction (Figure 2b) to simulate the use of mandibles for food
162 compression. We constrained nodal displacement in x, y, and z directions and applied a 1 N
163 load uniformly distributed among nodes in all simulations. We modeled the mandible cuticle
164 as an isotropic and linearly elastic material, setting Young's modulus as 2.75 GPa and the
165 Poisson's ratio as 0.3, based on measures from the cuticle of ant mandibles available in the
166 literature [57]. Given that we intended to investigate how variation in mandible morphology
167 affects the mechanical responses to the same loading demands in different biting conditions,

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3 168 the only source of variation for each biting simulation between species and workers was the
4 morphology of the mandibles. Therefore, we can test if some morphologies are better suited
5 169 morphology of the mandibles. Therefore, we can test if some morphologies are better suited
6 for specific biting conditions. We present FEA stress results based on Tresca failure criterion,
7 170 for specific biting conditions. We present FEA stress results based on Tresca failure criterion,
8 more suitable for brittle fracture, which determines an equivalent stress value under which
9 171 more suitable for brittle fracture, which determines an equivalent stress value under which
10 172 the material will possibly fail when subjected to combined load [58]. We used Abaqus 6
11 (Dassault Systèmes) to run the FEA simulations. Mandible 3D solid models are available on
12 173 (Dassault Systèmes) to run the FEA simulations. Mandible 3D solid models are available on
13 174 the supplementary material (Supplementary Material File S1).

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176 **Results**

177 *FEA simulations*

178 Stress distribution results are shown in Figure 3. Given that the volume of each
179 model varies, and that we used idealized loads and material properties, we chose not to
180 interpret absolute stress values. Instead, we will focus on qualitative differences among
181 simulations by rescaling the stress ranges based on a reference model to facilitate
182 comparisons between species, subcastes, and biting scenarios (Supplementary Material Fig.
183 S1). Therefore, relative differences in stress distribution between simulations indicate
184 mandibular biomechanical distinctions to assimilate loading conditions.

185

186 *Major worker mandibles*

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

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

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3 207 *hetschkoi* has a deeper groove near the *mm*, which acts as an important stress concentrator,
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5 208 mainly in strike scenarios on the *mm* (*ifg*, Fig. 3g). While *P. cf. lucretii* also shows stress
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7 209 concentration at the same region in this biting scenario, those stresses spread more
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9 210 extensively along the *if* (Fig. 3h), which suggests that its groove is shallow and does not act
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11 211 as a stress concentrator. The *ef* curvature also differs between species, but there are no
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13 212 substantial differences in stress dissipation patterns (Fig. 3c, d, g, h, k, l, o, p). The dissipation
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15 213 through the *ef* is more restricted to the articulation surroundings, given the robustness of the
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17 214 mandibular base, which could explain why there is not a conspicuous effect of the *ef*
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19 215 curvature in the stress dissipation pattern between species. Stresses were proportionately
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21 216 higher in the *P. cf. lucretii* mandible, through most mandibular regions and all biting
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23 217 scenarios, but the differences are more striking in pressure scenarios (Fig. 3l, p).

218 219 *Minor worker mandibles*

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221 There is a distinguished stress concentration around the more constricted region of
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223 the *if*, a trend that occurs mainly in strike simulations, especially when the load was applied
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225 on the *mm* (Fig. 3e, f). This constriction acts as a stress concentrator in minors due to their
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227 slender mandibles in comparison to majors. When the results of different species are
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229 compared, *P. cf. lucretii* simulations show proportionately higher stresses than *P. hetschkoi*
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231 in general (Fig. 3b, f, j, n), contrary to the expectation that minors mandibles would not differ
232
233 in mechanical performance. The overall lower stress levels found in *mm* strike simulations
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235 of the *P. hetschkoi* minor seems to reflect the presence of well-developed teeth along its *mm*.
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237 It is noticeable that the *mm* teeth absorb great levels of stress (Fig. 3e) so that their absence
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239 leads to higher stress levels along the mandible surfaces in strike simulations of *P. cf. lucretii*
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241 minor, as well as in majors of both species. The higher stresses along the *if* in *P. cf. lucretii*
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243 minor mandible, compared to *P. hetschkoi* minor mandible, draw attention to the mechanical
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245 limitations associated with worn mandibles, as is the case of the *P. cf. lucretii* minor mandible
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247 modeled, which can lead to behavioral switches in task performance along the worker
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249 lifetime.

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251 Regarding the biting scenarios, pressure in minors results in higher stresses on the
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253 mandibular internal and external faces of both species when compared to majors (Fig. 3i, j,
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255 m, n). As occurred in pressure scenarios for majors, stresses along the *if* concentrate near the
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238 base of the mandible, where the load was applied. However, in minors, the mandible base is
239 slender, which can explain why the mandibular surfaces in minors are proportionately more
240 stressed in pressure than in strike simulations.

For Review Only

241 Discussion

242 In this study, we apply FEA in mandibles of *Pheidole* workers to simulate different
243 biting scenarios and investigate how morphological differences in mandible morphology
244 reflect their responses to those bite loading demands. Our results demonstrate how the
245 mandible morphology of dimorphic workers can be optimized for particular tasks and draws
246 attention to the role of specific mandibular regions or structures to deal with the stresses
247 generated by their bite. Most extant lineages preserve the primitive ant mandible shape,
248 which consists on a blade whose masticatory margin possess a row of teeth for cutting and
249 grasping [59], and that can be divided into two components, a basal thick stem, and a distal
250 triangular blade [32]. Our results indicate that the increased thickness of the mandible basal
251 region may conform to the high loading demands experienced by the mandibular articulations
252 with the head. Most of the stresses generated on the apical tooth dissipate along the external
253 margin towards the mandibular base, in both species and subcastes, avoiding the spread of
254 considerable stresses through the more delicate mandibular surfaces. In strike simulations on
255 the masticatory margin, the presence of well-developed teeth results in stresses being
256 concentrated on the teeth instead of spreading through the internal face of the mandible.
257 Majors of *Pheidole*, in which the masticatory margin is toothless, show high levels of stress
258 in the masticatory margin concavity that is not entirely absorbed in this region. Interestingly,
259 they have a deeper groove on their mandible internal face, especially *P. hetschkoi*, which
260 helps to concentrate stresses near the more robust masticatory margin instead of spreading
261 through the internal face of the mandible. Although alleviating the stress level in the
262 mandibular articulations, such stress concentration can be harmful in cases in which the
263 structure is submitted to cycles of loading, leading to structural failure due to material fatigue
264 [60].

265 An important aspect of *Pheidole* mandibular morphology to bite mechanics is the
266 role of the trulleum and the canthellus on stress concentration. The trulleum is a concavity
267 near the *dma* that is delimited by a cuticular ridge called canthellus, a configuration that is
268 present only in some myrmicine ants [30]. The function of the trulleum was hitherto unknown,
269 although it was suggested that it could act as an additional stabilization of the mandible [32].
270 Here we demonstrate for the first time the importance of the trulleum and the canthellus to
271 assist in stress concentration along the *dma*, avoiding the spread of stresses through the more

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3 272 delicate mandibular surfaces. This is an interesting discovery, given that the *dma* seems to
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5 273 concentrate higher stresses in general than the *vma*. Given the suggested functional role of
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7 274 those mandibular regions outlined by our results, it would be interesting to investigate the
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9 275 biomechanical responses of mandibles that lack the development of this structure to
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11 276 understand how stresses dissipate from *dma* without those important stress concentrators,
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13 277 especially in ant species with similar loading demands as *Pheidole* mandibles. Although
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15 278 many ant lineages share the common mandible triangular shape as *Pheidole* species [32],
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17 279 other subtle morphological characteristics could assist in stress concentration (e.g., the
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19 280 cuticular thickness around this region and the mandible curvature pattern). Differences in
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21 281 mandible use and diet can also influence the amount of stress in the mandibular articulations
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23 282 (e.g., a diet rich in liquid food represents much lower bite loading demands than one
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25 283 composed of seeds or arthropods).

24 284 Our results also underscore how more robust major mandibles are better suited to
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26 285 deal with pressure biting than the minors' slender mandibles, which surprisingly show higher
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28 286 performance in strike scenarios. These results agree with the specialized roles played by
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30 287 major workers in the colony. The behavioral repertoire of major workers is particularly
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32 288 limited, being frequently restricted to defense and/or food processing [17,22]. Indeed, when
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34 289 minors are experimentally removed from the colony, major workers take over many of their
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36 290 tasks, although with decreased efficiency [22,61]. Major mandibles meet the demands to deal
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38 291 with the processing of hard food items through pressure, with their toothless masticatory
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40 292 margin spreading bite forces evenly around the food item. Seed consumption is considered
41
42 293 an important aspect in the evolution of several myrmicine genera, such as *Pheidole*,
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44 294 *Pogonomyrmex*, and *Solenopsis* [21,62]. However, the influence of granivory on
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46 295 morphological evolution, especially regarding the dimorphism in the *Pheidole* worker caste,
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48 296 is still poorly known [24]. Here, we demonstrate for the first time how ant mandible
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50 297 morphology can be tuned to deal with the mechanical demands of processing hard food items,
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52 298 such as seeds and arthropod cuticles, through the better performance of majors' mandibles in
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54 299 pressure biting conditions. Also, mandibles of *P. hetschkoi* majors show an even better
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56 300 performance in pressure bite than *P. cf. lucretii*, suggesting that majors of *P. hetschkoi* can
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58 301 deal better with harder food items than *P. cf. lucretii*. These results may lead to the possibility
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60 302 of food partitioning among *Pheidole* coexisting species and agree with the habit of seed

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3 303 consumption by *P. hetschkoi*, which demands higher bite forces and consequently leads to
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5 304 higher stress levels on the mandibles.

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7 305 In general, *Pheidole* minor mandibles show a more serrated and sharpened *mm*, with
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9 306 well-developed teeth, whereas majors have mandibles that are blunter and that show broader
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11 307 *mm* [39]. However, the particular specimen of *P. cf. lucretii* minor worker included in our
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13 308 study showed high levels of teeth wear, allowing us to assess the consequences of teeth wear
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15 309 on bite loadings. Teeth concentrate the forces generated by the masticatory muscles on
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17 310 smaller areas, with the potential to improve the initiation of fracture in the gripped object
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19 311 [63]. The importance of teeth on task efficiency was demonstrated for leaf-cutting ants, in
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21 312 which workers specialized to cut leaves switch to carrying them once their teeth are worn to
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23 313 a certain degree, reducing their cutting efficiency [64]. In *Pheidole*, minors perform a wide
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25 314 range of tasks in the colony [17,22], but information on the role of teeth wear on the
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27 315 probability of task switch in minors is scarce. Here we demonstrate the possible mechanical
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29 316 consequences of teeth wear in ant mandibles, comparing the relative amount of stress
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31 317 generated during masticatory margin strike simulations in *P. hetschkoi* and *P. cf. lucretii*
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33 318 minors. Our results indicated that *P. cf. lucretii* has relatively higher stresses than *P.*
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35 319 *hetschkoi*, mainly along its internal face of the mandible, which drives higher stresses at the
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37 320 mandibular articulations with the head. Further studies on task allocation and mandible
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39 321 morphology in dimorphic ant species can address if teeth wear generates task switch, and
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41 322 biomechanical studies can reveal how teeth wear reduces task efficiency [64]. Also important
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43 323 is to understand if cuticle hardening by heavy metal bioaccumulation in the mandible
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45 324 masticatory margin [57,65] could help mitigate the stress levels reaching the mandibular
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47 325 faces and articulations, as suggested for genital damage in bush crickets [66].

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49 326 The morphological evolution of *Pheidole* might be strongly driven by differences in
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51 327 size [23], which tends to evolve at higher rate than shape [18,25,27]. More recently, studies
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53 328 applying geometric morphometrics approaches validated the prominence of size to explain
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55 329 the morphological disparity in the genus but also pointed to different evolutionary rates and
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57 330 levels of integration between head and mesosoma shape and size [27,28]. *Pheidole*
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59 331 morphological diversification seems to be very constrained [23], in contrast to their
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332 ecological disparity [18,67], as reflected in the widespread distribution of the genus
333 throughout most of the terrestrial ecosystems [19]. Field observations demonstrate that,

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3 334 despite the relative morphological resemblance in *Pheidole* species, they can show
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5 335 considerable ecological and behavioral diversity [68,69]. Here we demonstrate that even
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7 336 small morphological differences in mandible shape between species can lead to
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9 337 biomechanical specialization, mainly in major's food processing capacity, as suggested by
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11 338 descriptions of the mandibular morphology variation in *Pheidole* species [39]. This
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13 339 biomechanical specialization can expand the diet range of species and contribute to food
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15 340 partitioning [20,70,71], decreasing the degree of competition and allowing for species
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17 341 coexistence [72].

17 342 Our results provide a biomechanical basis to understand how mandible
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19 343 morphological evolution can improve task specialization in polymorphic ants and help
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21 344 developing a general understanding of form-function relationships in ant mandibles.
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23 345 Morphological polymorphism in the worker caste can expand the range of prey items that a
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25 346 species can handle, as demonstrated for some army ants in the genus *Eciton* [11,12]. In the
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27 347 highly polymorphic genus *Cephalotes*, which together with *Procryptocerus* is *Pheidole*'s
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29 348 sister lineage, some workers have the head modified into a flat surface used to obstruct and
30
31 349 close the nest entrance, protecting the colony against invasion [73]. In some fire ants, such
32
33 350 as *Solenopsis geminata* (Fabricius), the degree of worker polymorphism is associated with
34
35 351 higher levels of division of labor, with major workers being specialized in seed milling
36
37 352 [62,74]. Division of labor in leaf-cutting ants is associated with morphological distinctions
38
39 353 among worker mandibles, as demonstrated for the polymorphic genus *Atta* [41]. In addition,
40
41 354 refined morphological descriptions of *Pheidole* workers mandibles suggest that differences
42
43 355 in the masticatory margin can improve task specialization [39].

41 356 Although the role of worker polymorphism for division of labor in ants is well
42
43 357 established [8], we show that, by applying biomechanical approaches we can advance our
44
45 358 understanding about the functional role of morphological disparity, as demonstrated here for
46
47 359 *Pheidole* workers. Polymorphic ant lineages are ideal models to investigate form-function
48
49 360 relationships, and the morphological differentiation of their mandibles should be studied in
50
51 361 detail, given the importance of this structure to worker interactions with the environment.
52
53 362 Future studies can contribute to our knowledge on the evolution of ant mandibles morphology
54
55 363 by investigating the role of metal bioaccumulation [75-77] and the consequent cuticle
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57 364 hardening [57,65] in the mechanical response of mandibles to bite loading demands.

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3 365 Additionally, biomechanical approaches investigating the variation in bite force in
4 366 morphologically polymorphic ant lineages can provide valuable information for the evolution
5 367 of task specialization [39], and to understand how mandible morphology can be optimized to
6 368 deal with powerful or fast movements [38].
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11 370 **Authors' contributions**

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14 371 ACF and EPE collected the raw data; CLK, ACF, MAA and MRP contributed on the study
15 372 design; CLK, ACF and MAA contributed on data analysis; CLK and MRP drafted the
16 373 manuscript; ACF, EPE and MAA critically revised the manuscript; MRP coordinated the
17 374 study.
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23
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26 378 OIST Imaging section for access to the CT scanner.
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30 379 **Data accessibility**

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32 380 Three-dimensional reconstructions of the *Pheidole* workers mandibles used in the Finite
33 381 Element Analysis simulations are available as Supplementary Material File S1 at “figshare
34 382 repository”.
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37

38 383 **Ethics**

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40 384 This article does not present research with ethical considerations.
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42

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610 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)
Major	<i>P. hetschkoi</i>	2.35	0.023	449488	5.30876	1.10	1.84
	<i>P. cf. lucretii</i>	1.011	0.023	278634	4.49981	0.85	1.18
Minor	<i>P. hetschkoi</i>	0.503	0.0035	881691	3.89985	0.65	0.80
	<i>P. cf. lucretii</i>	0.25	0.0035	392790	4.04989	0.47	0.58

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3 612 **Figure captions**

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5 613 Fig. 1. 3D models of *Pheidole* workers heads. *Pheidole hetschkoi* (a) major and (b) minor
6 614 worker. *Pheidole cf. lucretii* (c) major and (d) minor worker.

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10 616 Fig. 2. Loaded and constrained regions in strike (a) and pressure (b) biting simulations. In (a)
11 617 and (b), black arrows indicate the direction and region of load, and dashed lines enclose the
12 618 constrained regions for each simulation. *al*: atala; *at*: apical tooth; *bm*: basal margin; *dma*:
13 619 dorsal mandibular articulation; *ef*: external mandibular face; *em*: external margin; *if*: internal
14 620 face of the mandible; *mm*: masticatory margin; *vma*: ventral mandibular articulation; *Omd1*:
15 621 muscle *craniomandibularis internus*.

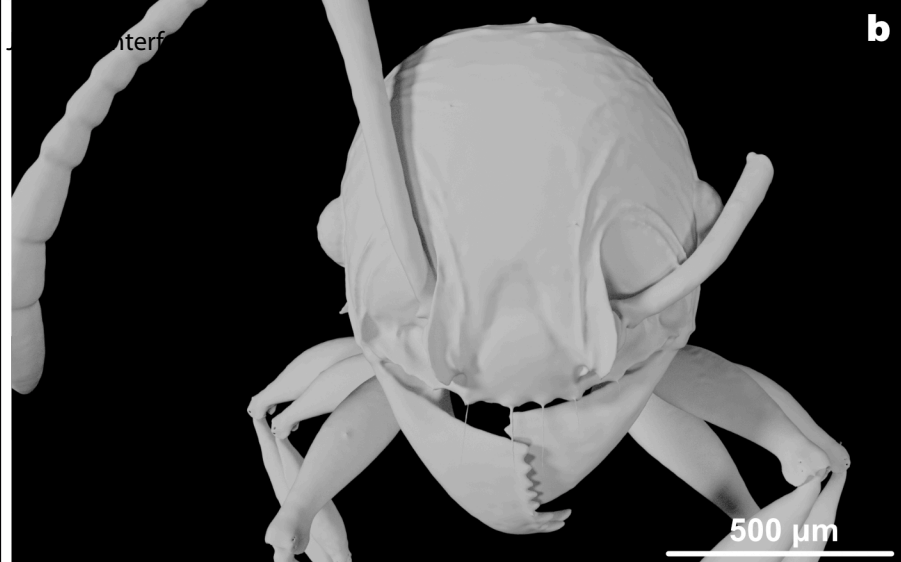
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17 622
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19 623 Fig. 3. Tresca stress results (rescaled to range between 0-1) for the four biting scenarios
20 624 (rows), from minors and majors of both *Pheidole* species (columns). Each letter depicts a
21 625 distinct simulation. Color represents a proportional value of stress in relation to the maximum
22 626 value considered for each simulation, indicated as 1.00, and grey represents extremes values
23 627 above the maximum considered. *at*: apical tooth; *ca*: canthellus; *dma*: dorsal mandibular
24 628 articulation; *ef*: external mandibular face; *em*: external margin; *if*: internal face of the
25 629 mandible; *ifg*: groove on the internal face of the mandible; *mm*: masticatory margin; *tr*:
26 630 trulleum; *vma*: ventral mandibular articulation.

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a

500 μ m



b

500 μ m



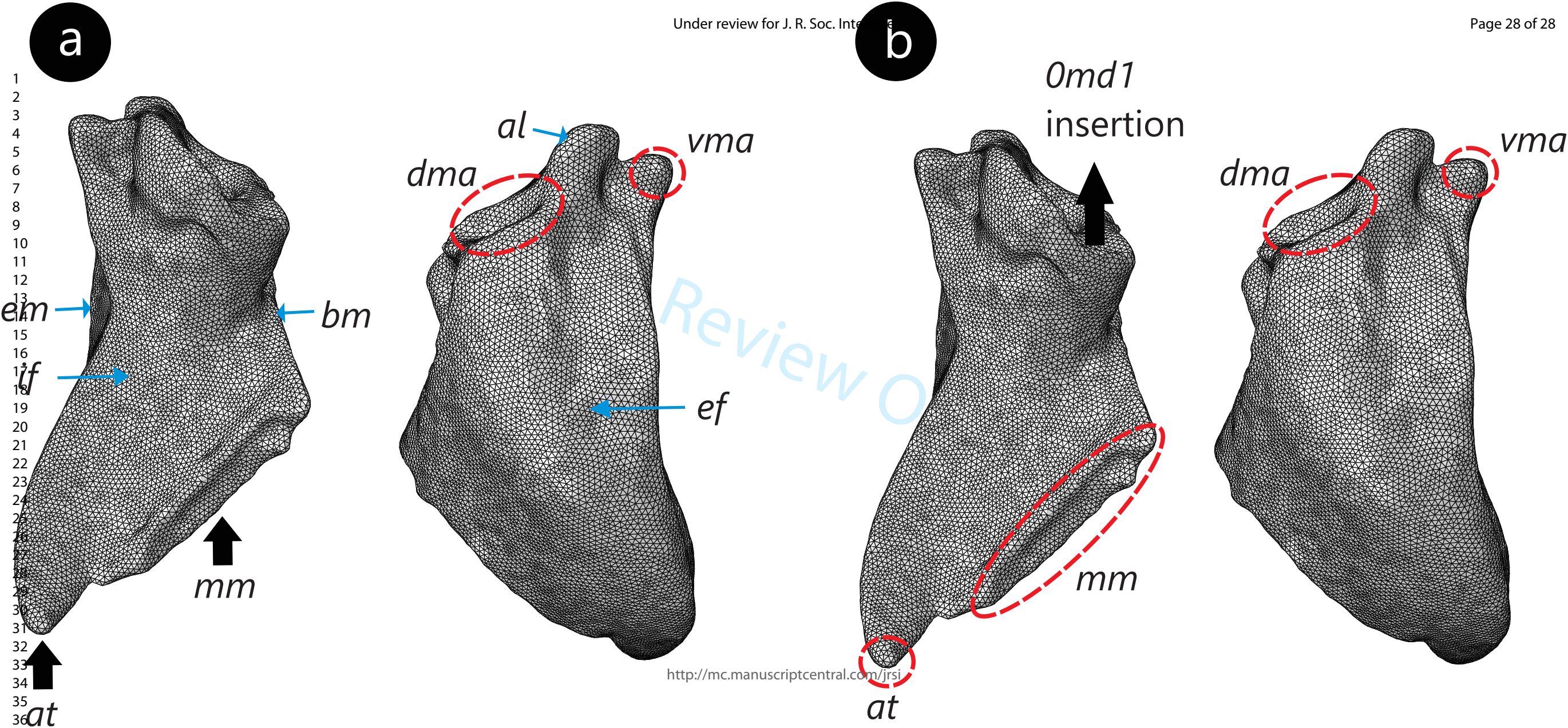
c

500 μ m



d

500 μ m



a

b

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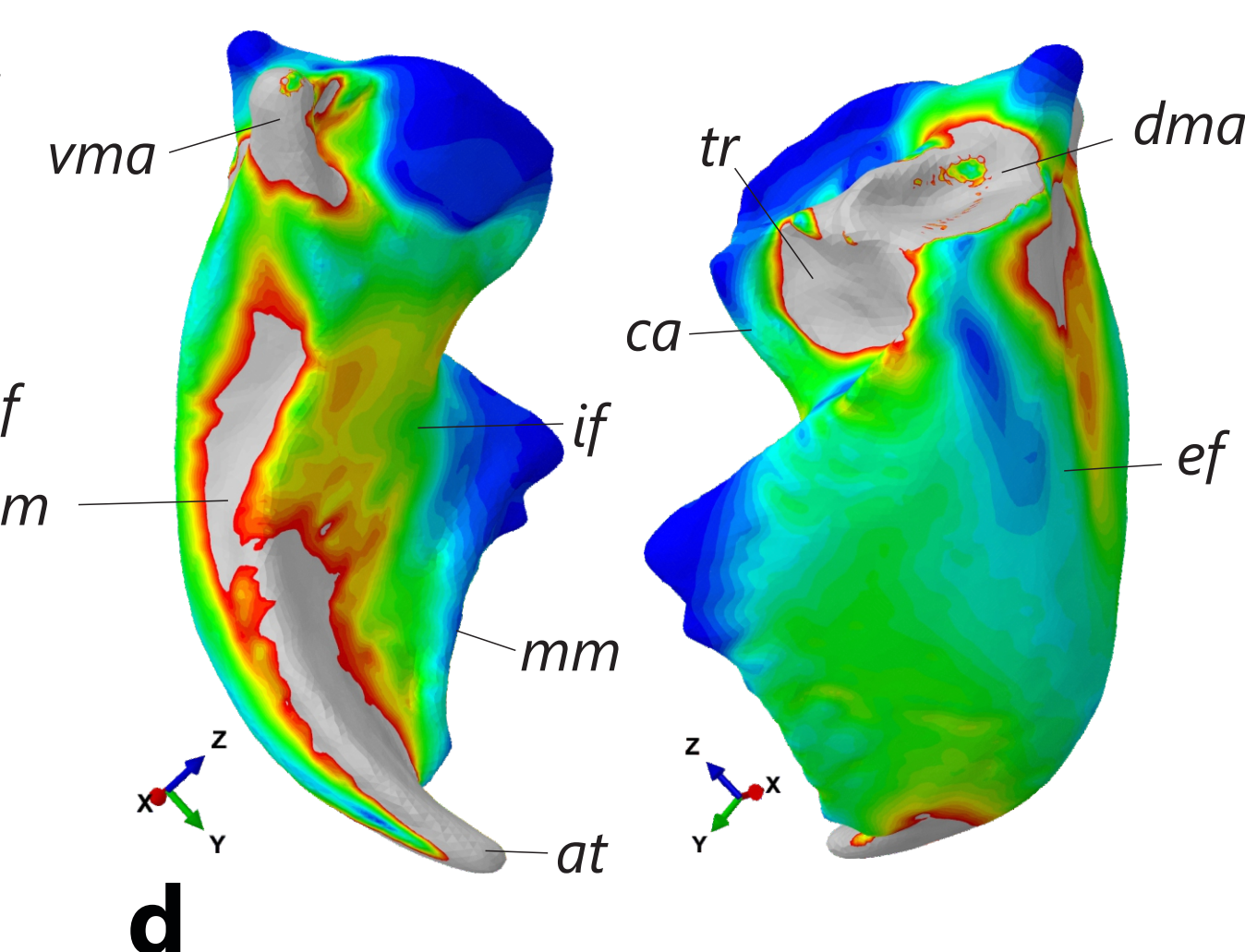
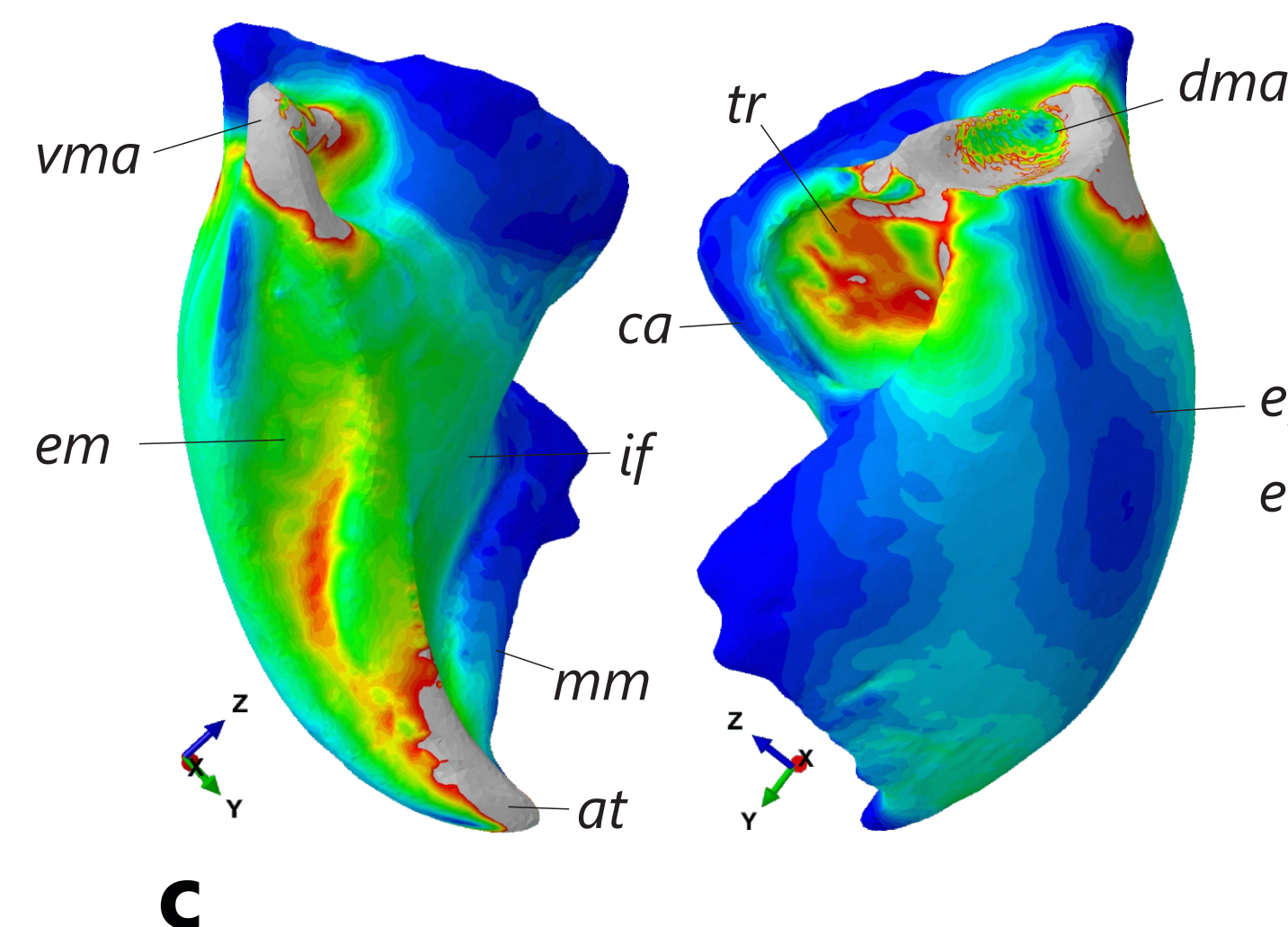
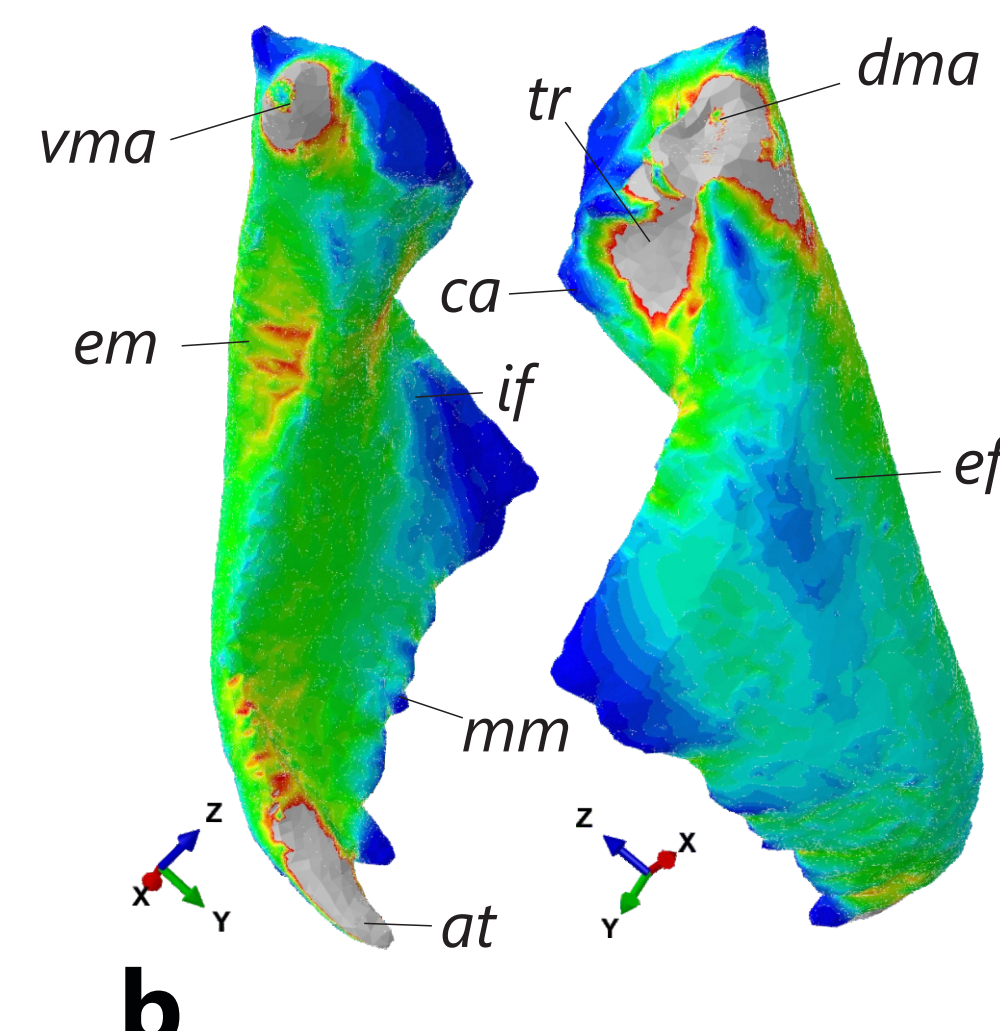
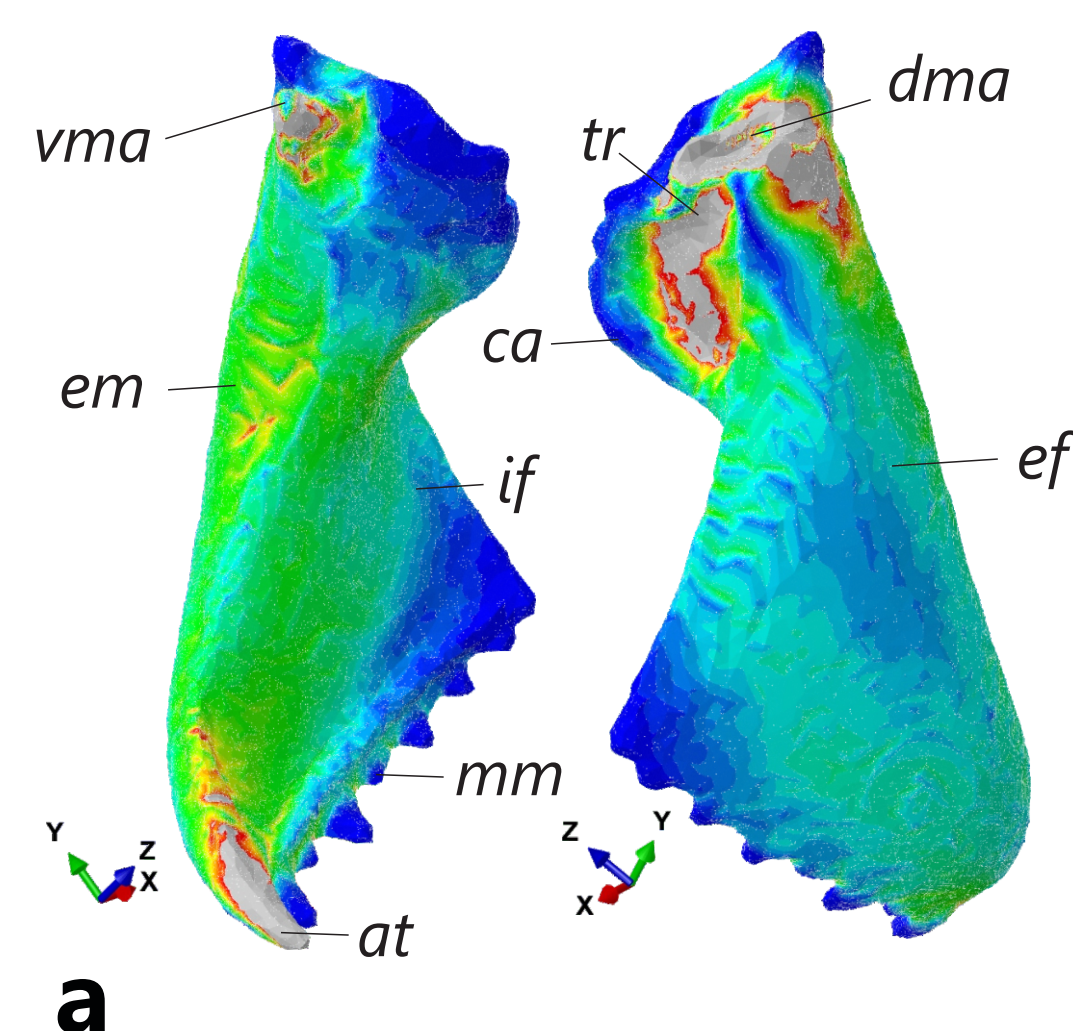
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P. hetschkoi

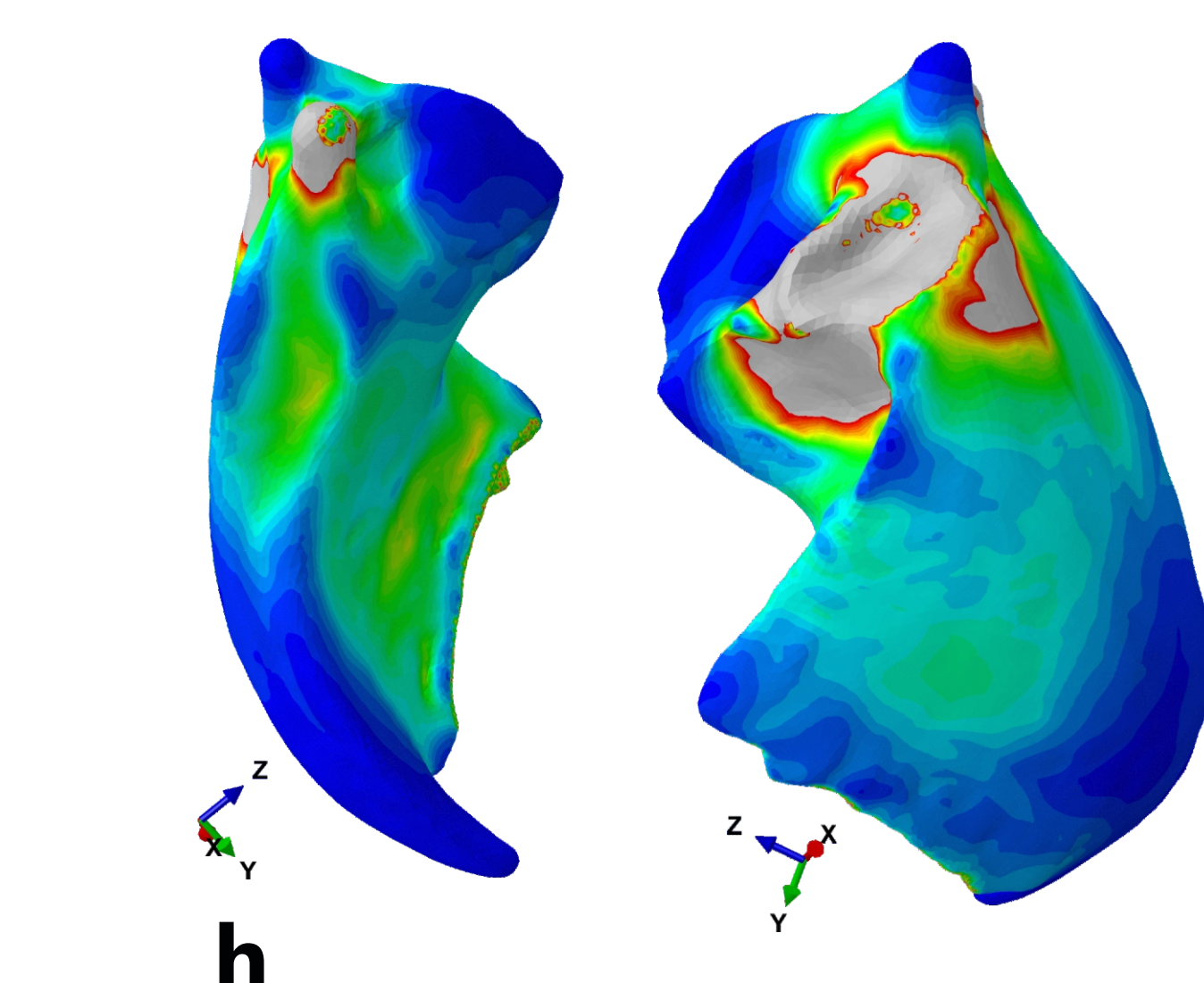
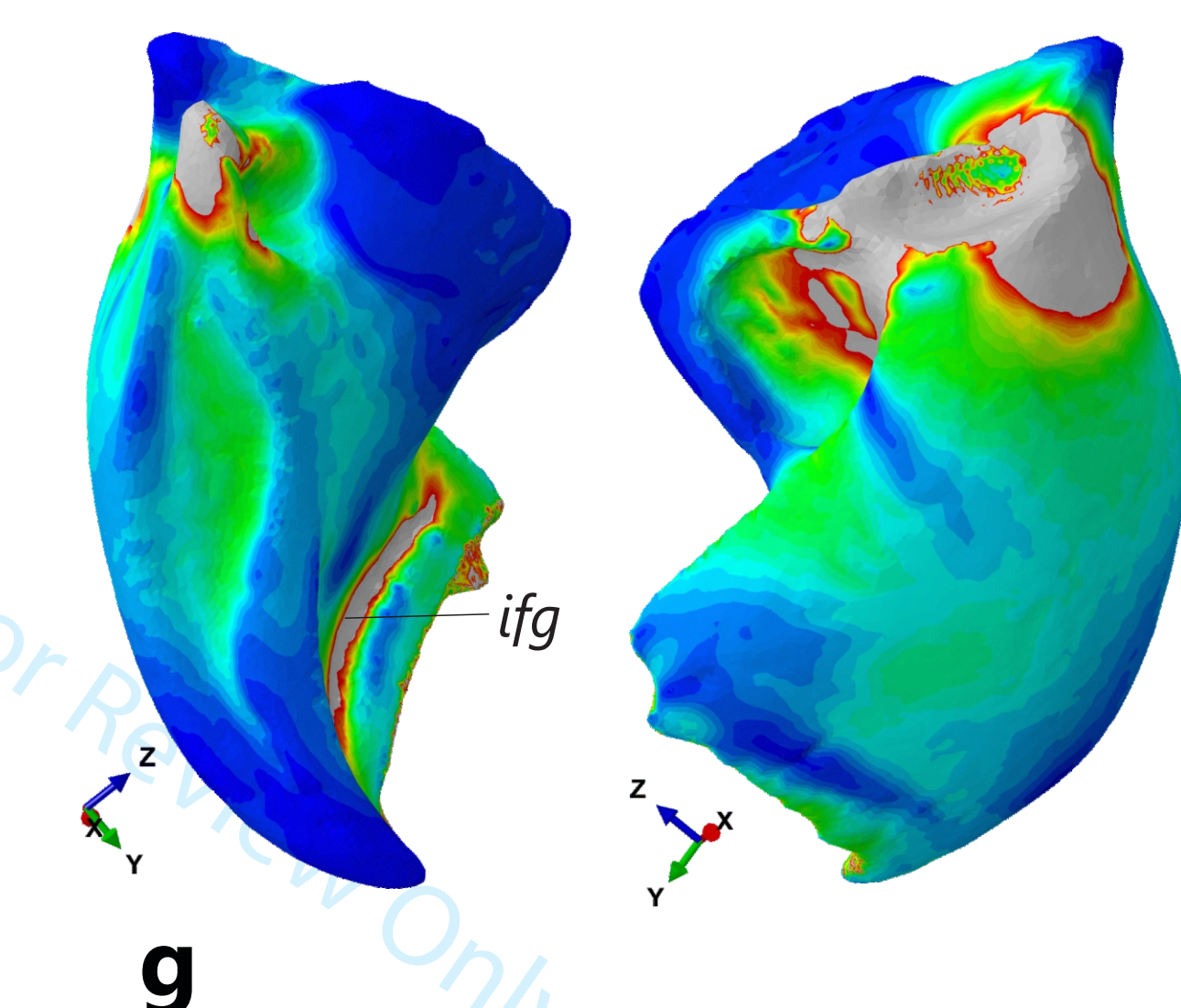
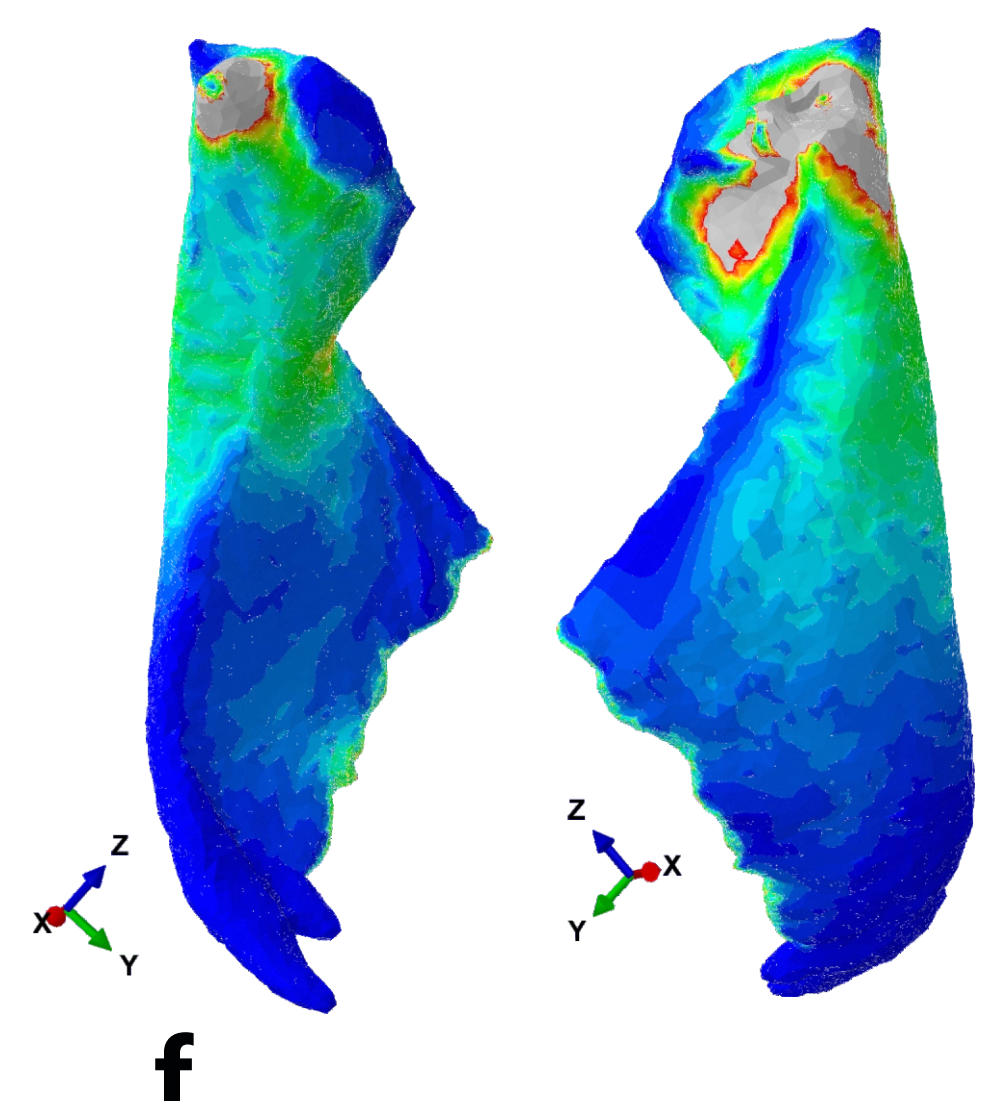
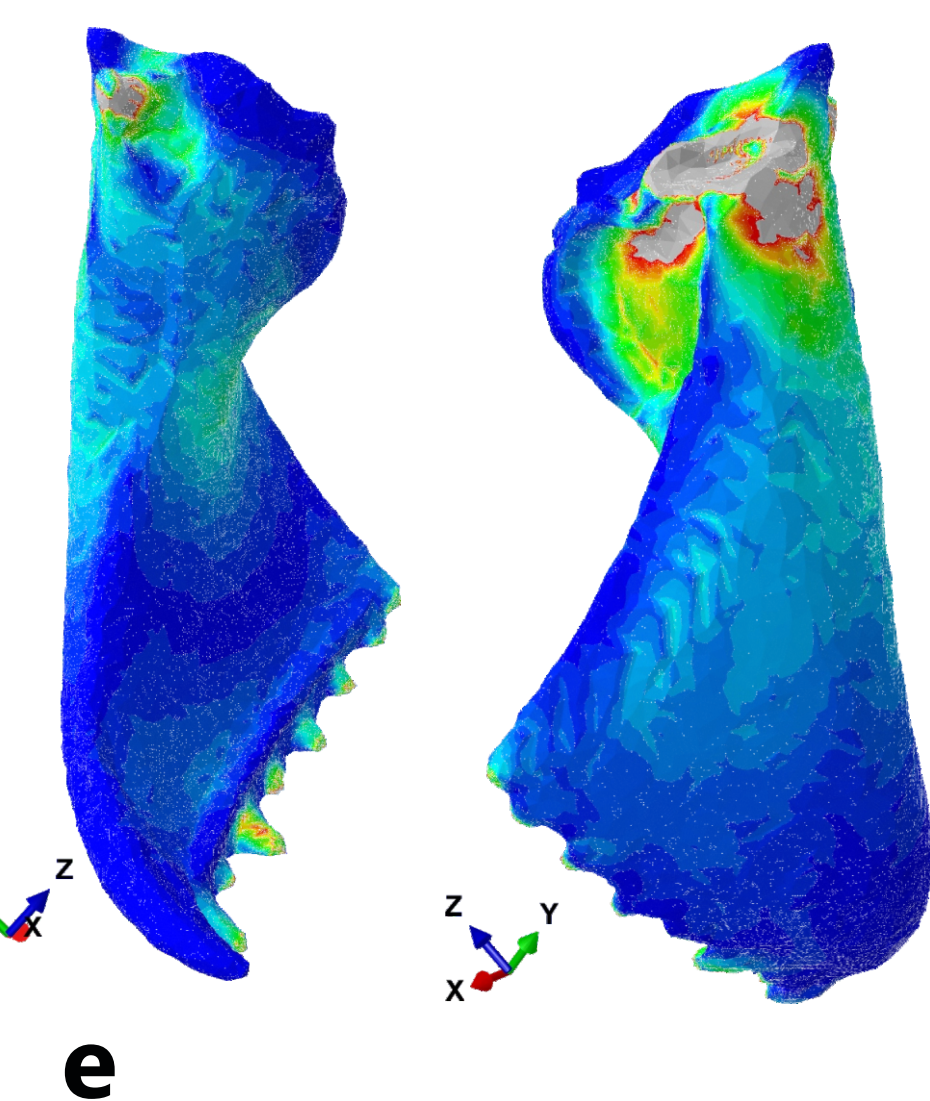
P. cf. lucretii

P. hetschkoi

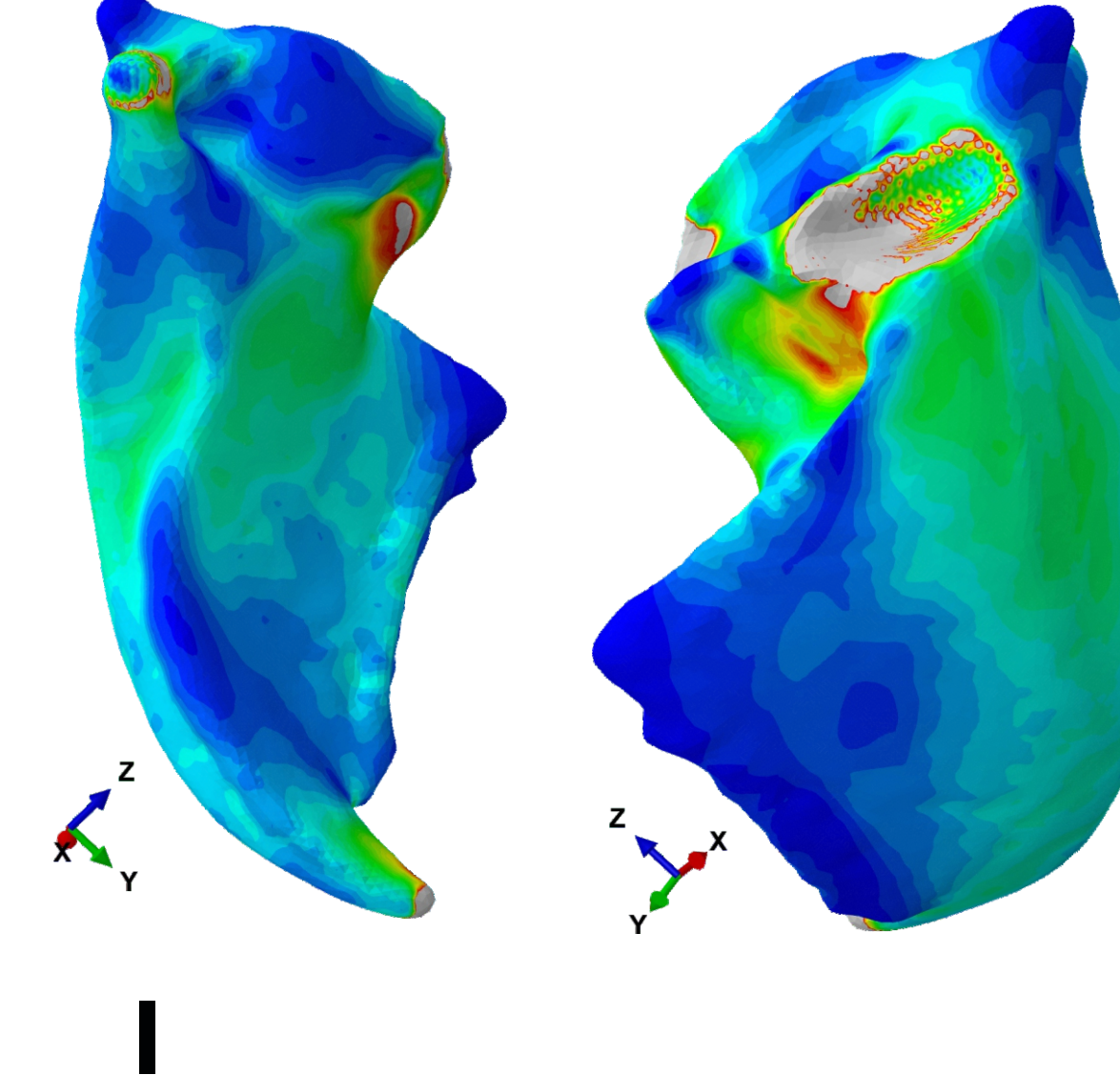
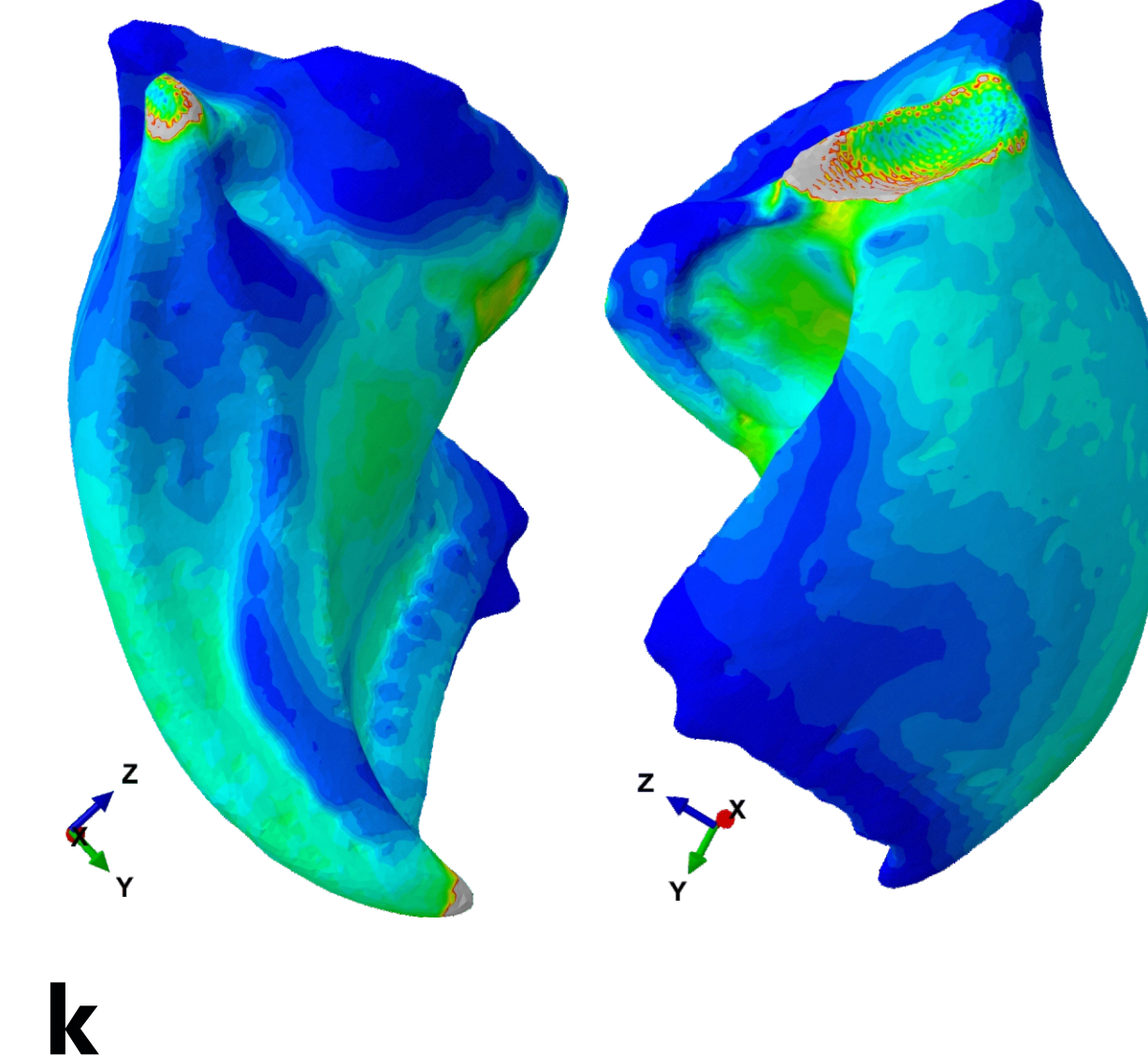
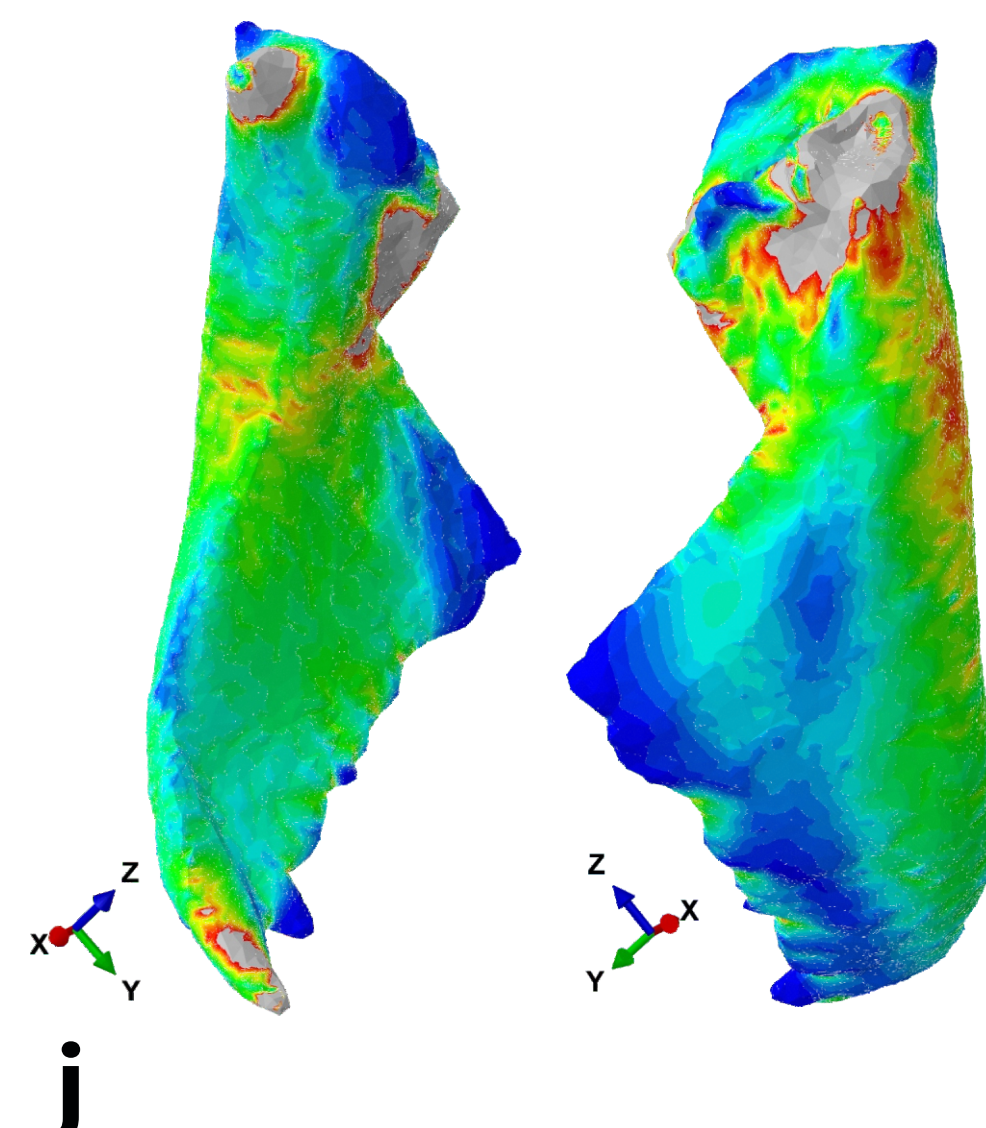
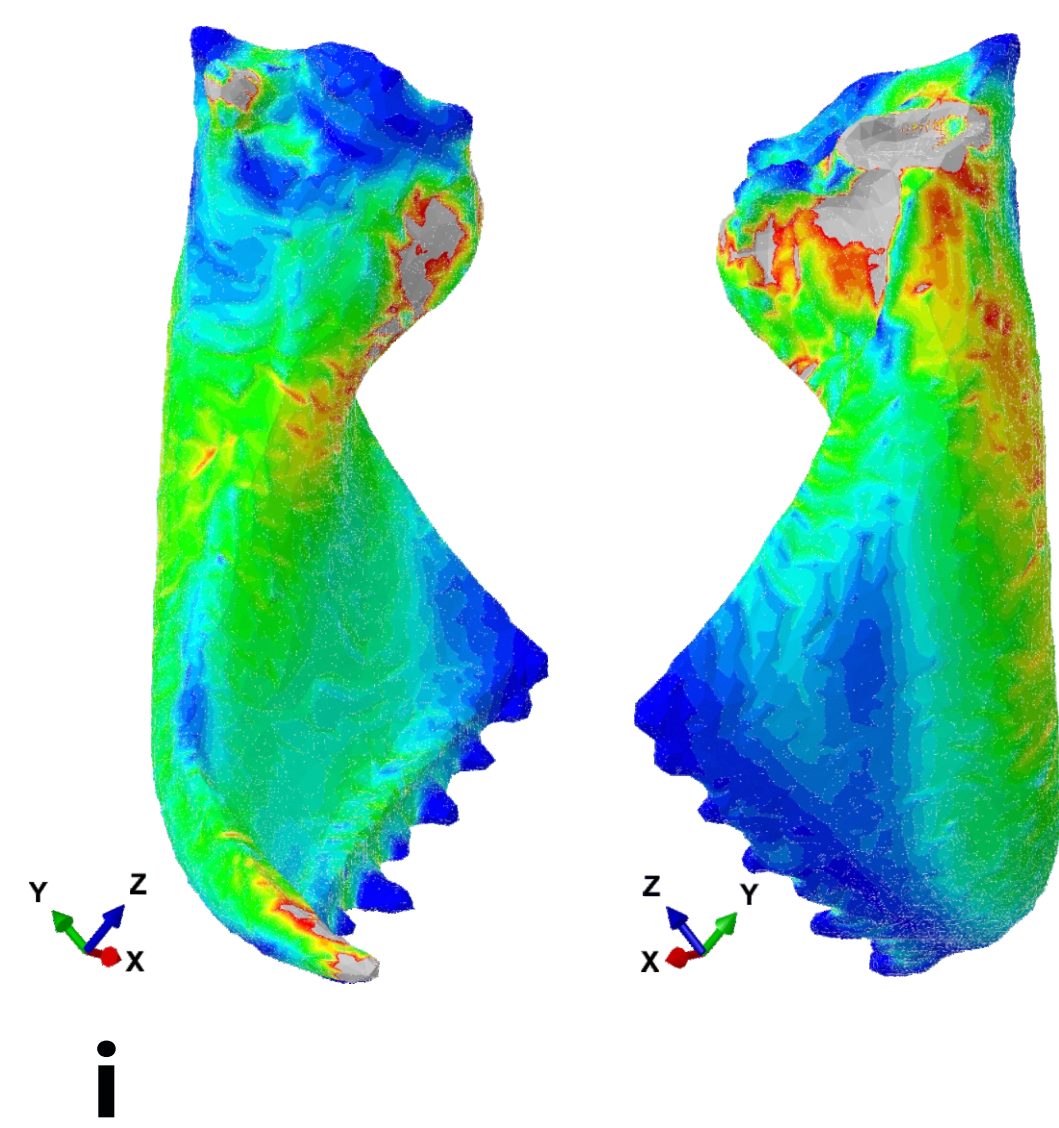
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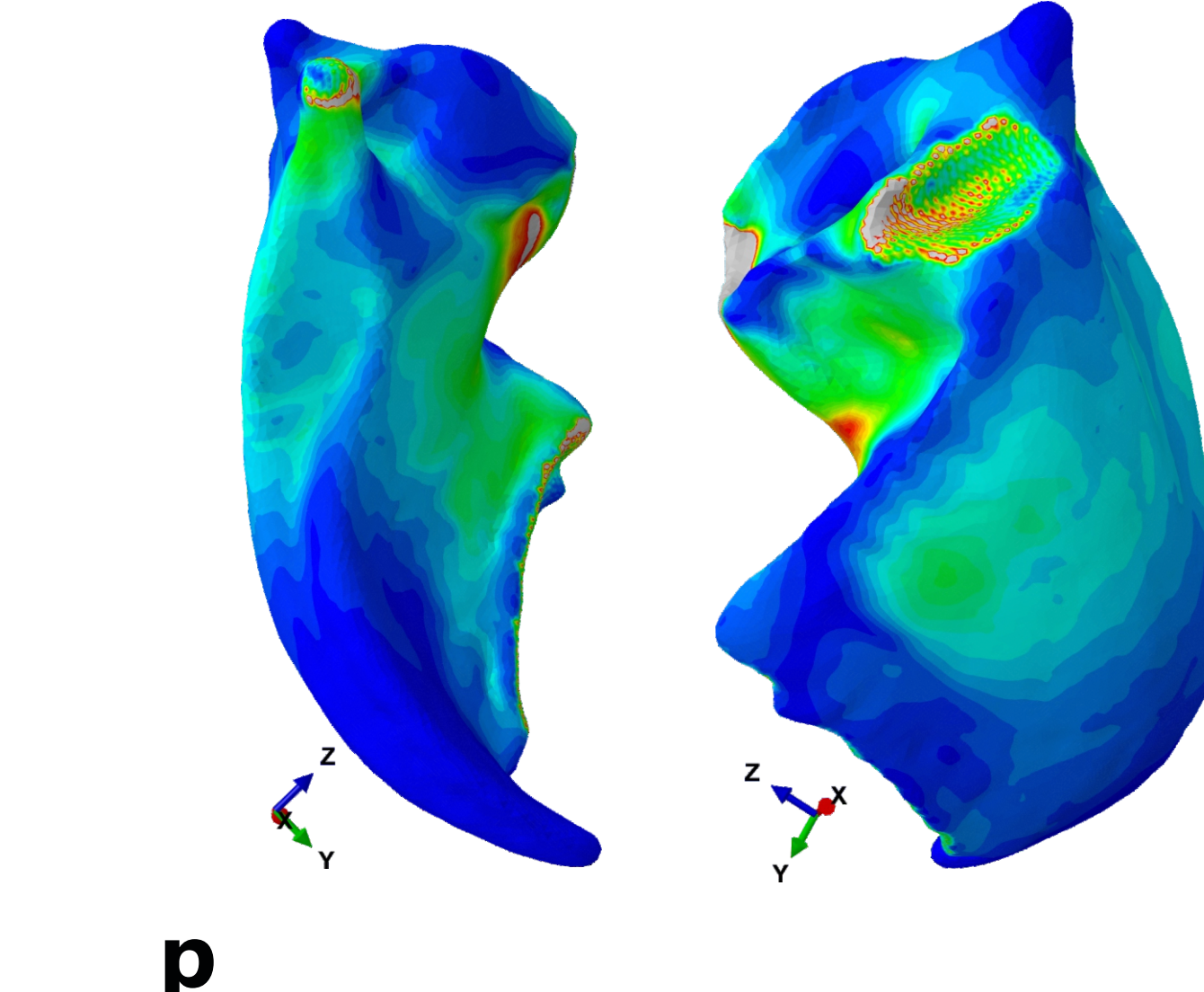
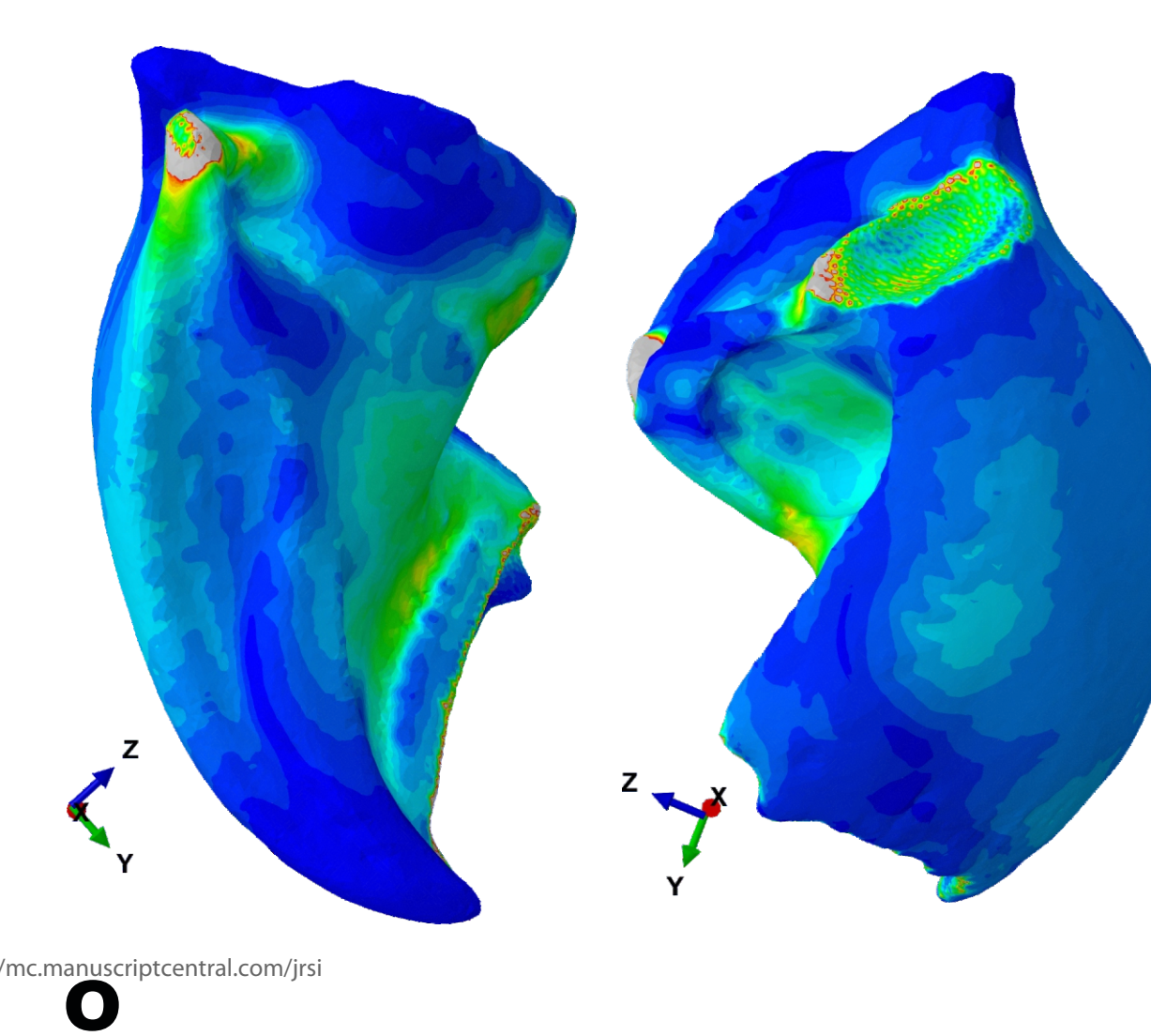
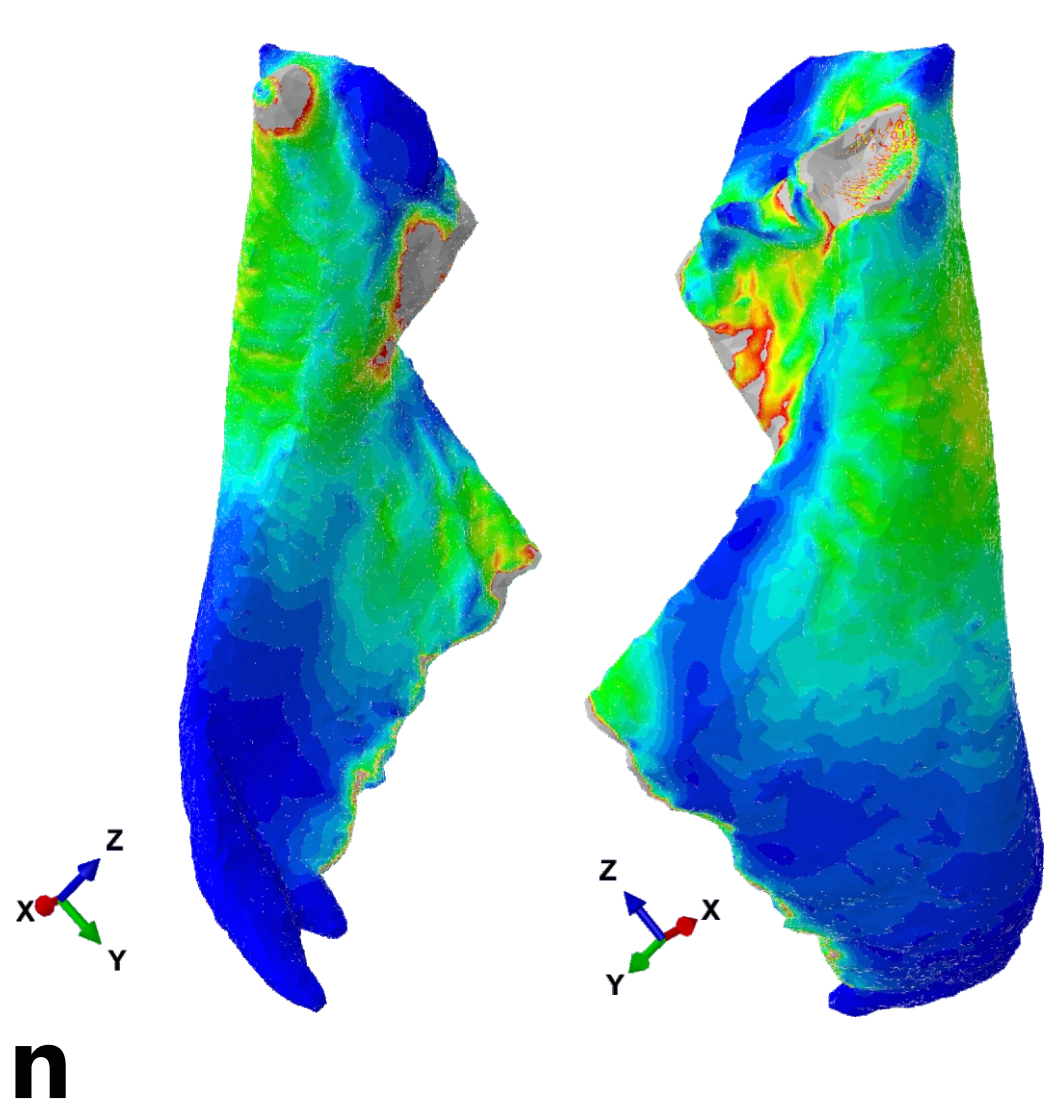
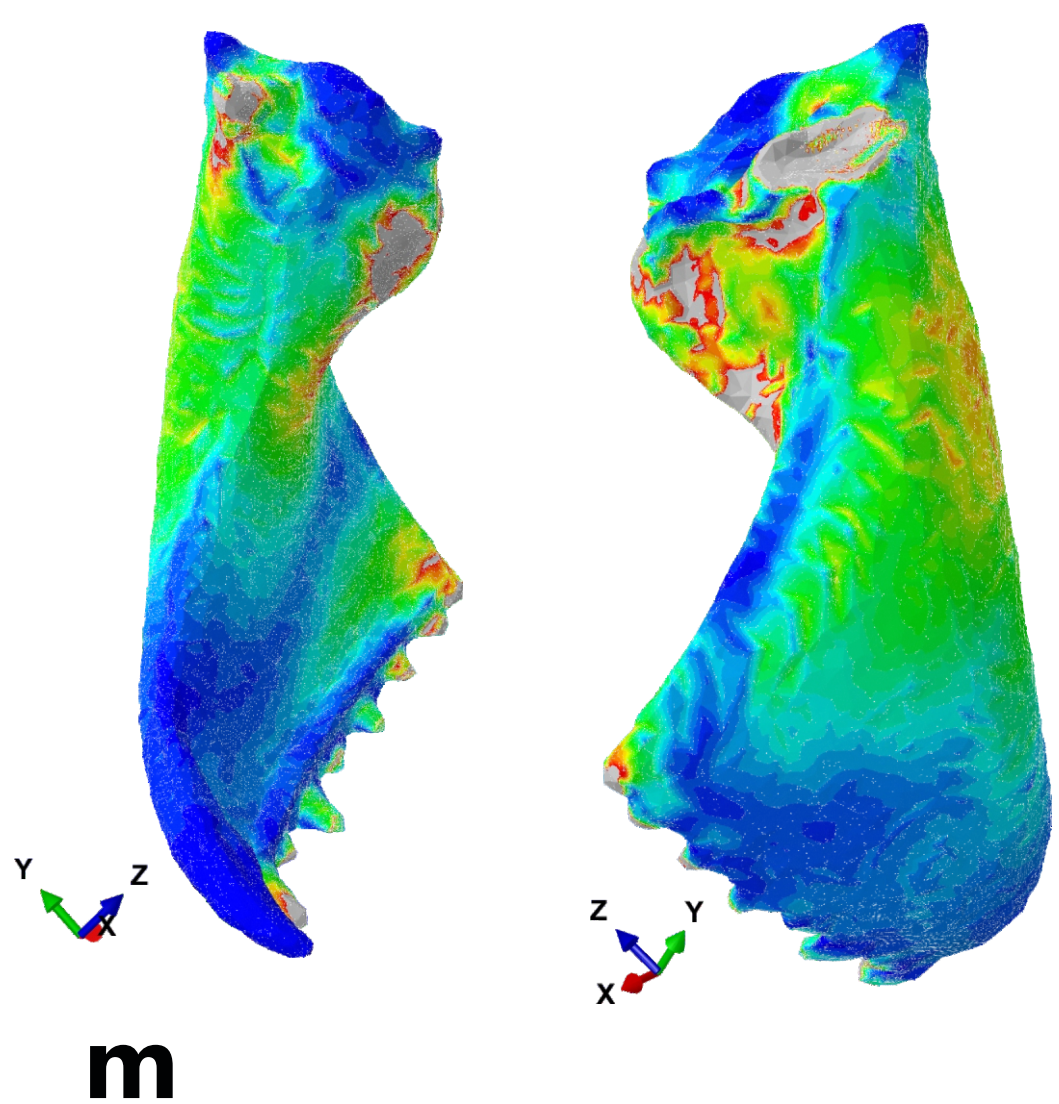
Apical strike



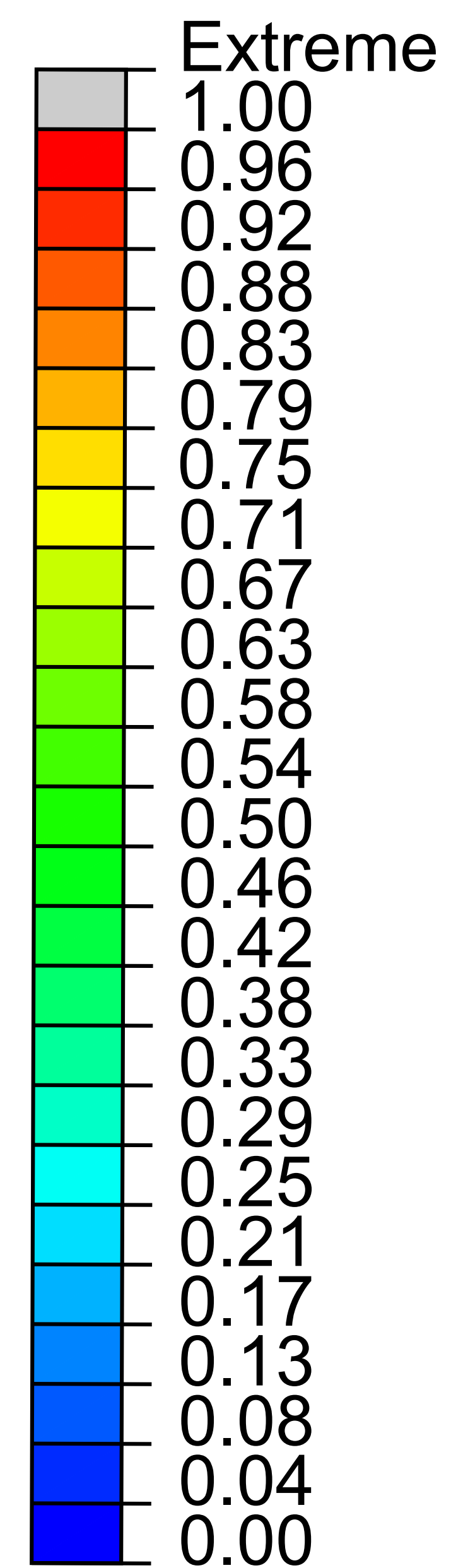
Masticatory margin strike



Apical pressure



Masticatory margin pressure



S, Tresca