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1 **Minute workers and large soldiers in the subterranean ant *Carebara perpusilla*:**
2 **musculoskeletal consequences of Haller's rule in the thorax**

3

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11

12 **Abstract**

13 Many organismal traits vary with body size, often reflecting trade-offs in the face of size-dependent
14 constraints. For example, Haller's rule, the allometric pattern whereby smaller organisms have
15 proportionally larger brains, can have carry-on effects on head design as the brain competes for space
16 with other structures. Ant species with polymorphic worker castes are interesting cases for helping us
17 understand these allometric effects. Here, we examine the effects of miniaturization on the ant power
18 core, the mesosoma (thorax), with particular attention to how the scaling of nervous system structures
19 affects the skeletomuscular elements involved with load bearing and locomotion. Using X-ray computed
20 microtomography (microCT), we studied the thorax of *Carebara perpusilla*, an African ant species that
21 has minute workers (1.5mm-long) and larger soldiers (3.0mm-long), allowing strong intraspecific
22 comparisons. We find that the thoracic nervous system is relatively larger in minute workers, similar to
23 Haller's rule, with consequences on the skeletomuscular organisation. Minute workers have relatively
24 smaller petiole muscles and indirect head muscles, but relatively larger external trochanter muscles and
25 direct head muscles. We link these allometric trade-offs to miniaturization and division of labor, and
26 discuss how thorax design underlies the success of minute ants.

27

28 **Keywords:** microCT, Formicidae, square-cube law, miniaturization, cuticle thickness

29 **1. Introduction**

30 Ant workers are well known for their ability to carry heavy objects or food items multiple times
31 their own weight. This ability is essential because ants are central place foragers: workers bring food to a
32 perennial nest to share it with nestmates instead of eating on the spot. The carrying power of ants lies in
33 their thorax – usually referred to as ‘mesosoma’ in ants because in Apocrita the three thoracic segments
34 are fused with the first abdominal segment, called ‘propodeum’ – which houses muscles that control the
35 head, legs and abdomen, necessary for foraging and locomotion. Ant workers, in contrast to queens and
36 other winged Hymenoptera, lack wing muscles, that normally fill 40-50% of the thorax volume, and
37 instead other muscles expanded to the vacant space (Keller et al., 2014; Peeters et al. 2020).

38 Miniaturization is the evolution of extremely small body size within a lineage (Hanken and
39 Wake, 1993). This phenomenon is widespread in insects and has driven the evolution of the smallest
40 metazoans (parasitoid wasps, see Polilov, 2015 for examples) as well as the evolution of ants. Indeed,
41 flight loss alleviated constraints on body size in worker ants, thus permitting miniaturization in most
42 genera, contrary to social bees and wasps (Peeters and Ito, 2015). During 140 Mya of evolution,
43 relatively few species of ants became large, e.g. in *Dinoponera*, 30mm, 8 spp (Lenhart et al., 2013),
44 *Paraponera*, 25mm, 1 sp (AntWeb, 2022), and *Dinomyrmex*, 28mm, 2 spp (AntWeb, 2022). In contrast,
45 workers became minute – body size below 2mm – in most extant species (Peeters and Ito, 2015), e.g. in
46 Myrmicinae genera *Monomorium*, 400 spp, *Carebara*, 250 spp, and *Pheidole*, 1100 spp (measured from
47 AntWeb pictures, AntWeb, 2022). Miniaturization brings many benefits to ants, such as opening up new
48 interstitial niches and reducing the cost and development time of individuals (Peeters and Ito, 2015).
49 However, miniaturization is more than a simple downscaling of individuals as the size of some organs
50 changes allometrically with body size. A famous example is Haller’s rule brain-body allometry, defined as
51 the brain getting relatively bigger with decreasing body size (Rensch, 1948). This rule has been validated
52 empirically in ants (Cole, 1985; Seid et al., 2011) as well as many vertebrate and invertebrate taxa
53 (reviewed by Eberhard and Wcislo, 2011). Moreover, miniaturization involves a reduction of sensory

54 organs like the eyes, and smaller ants - with less ommatidia - have a lower spatial resolution and
55 contrast sensitivity (Palavalli-Nettimi and Narendra, 2018; Palavalli-Nettimi et al., 2019). Nonetheless,
56 how vital organs and muscles scale with miniaturization inside the thorax is unknown. The internal
57 morphology of the thorax has only been studied in large ants that are not representative of the majority
58 of ants (Liu et al., 2019; Peeters et al., 2020; Aibekova et al., 2022). The strength of small ants is
59 commonly explained with physics and the square-cube law: the volume of the thorax is a cube
60 measurement (x^3) whereas muscle strength is proportional to its cross-sectional area, a square
61 measurement (x^2). The ratio between muscle strength and thorax volume ($x^2/x^3 = 1/x$) increases when
62 size (x) decreases, explaining why smaller animals can carry relatively heavier loads in general. However,
63 ants stand out in terms of load carrying, and a minute ant thorax must integrate functional organs (*e.g.*
64 from the nervous and digestive systems) along with numerous muscles in a tiny volume in order to
65 complete worker tasks.

66 Using X-ray computed microtomography, we compared the cuticle and internal thorax
67 morphology of *Carebara perpusilla* workers and soldiers, focusing on space allocation between neural
68 ganglia and muscles that move the head, legs and abdomen. *Carebara perpusilla* is a subterranean
69 African species with two wingless infertile castes – 1.5mm-long workers and 3mm-long soldiers (Figure
70 1, Khalife and Peeters, unpublished data) – allowing a powerful intraspecific comparison. Workers
71 perform brood care and foraging. They hunt soil microinsects such as springtails, as well as scavenge on
72 large insect carcasses that they bury with soil, retrieving only small meat pieces and hemolymph (Khalife
73 and Peeters, 2020). In contrast, soldiers rarely leave the nest: some guard the entrances while others
74 store food in their distended abdomen (repletes). Soldiers can be recruited to insect carcasses where
75 they use their powerful mandibles to cut through the cuticle, so that workers can access the meat.

76

77 **2. Material and Methods**

78 **2.1. Specimens and scanning**

79 Specimens of *C. perpusilla* were collected in a riverine forest on August 2016 in Gorongosa
80 National Park, Sofala province, Mozambique (S 19.00133 E 34.37730, ~ 100m elevation) and stored in
81 90% ethanol. Micro-CT scans were performed at the Okinawa Institute of Science and Technology
82 Graduate University (OIST, Japan) using a Zeiss Xradia 510 Versa 3D X-ray microscope operated with the
83 Zeiss Scout-and-Scan Control System software (version 11.1.6411.17883). One worker and one soldier,
84 selected randomly, were stained in a 2 M I₂E solution for 24 h (iodine dissolved in 99% ethanol, see
85 Gignac et al., 2016), and then transferred into microtubes filled with 99% ethanol for scanning. Scan
86 settings were selected to yield optimum scan quality. Full 360-degree rotations were based on 1601
87 projections. The resulting scan resolutions and parameter settings are provided in Table S1. Post-
88 imaging 3D reconstruction was done with the Zeiss Scout-and-Scan Control System Reconstructor
89 software (version 11.1.6411.17883), and the output files saved in DICOM format. While we performed
90 3D quantitative analyses on just one specimen per caste, we validated our observations with thorax
91 dissections and histological sections of other individuals.

92 **2.2. Segmentation**

93 Active labelling of voxels (volumetric pixels), *i.e.* segmentation, was performed from
94 reconstructed stacks using ITK-SNAP 3.6.0 (Yushkevich et al., 2006). For most structures, segmentation
95 was achieved semi-automatically with the ‘region competition’ algorithm followed by manual
96 segmentation to correct errors. Other structures such as the furcae were segmented manually every 5
97 slices and filled in using the ‘Interpolate’ tool. Identification of muscles and skeletal structures follows
98 Aibekova et al. (2022). The whole thorax cuticle, neural ganglia and oesophagus were segmented, and
99 muscles were segmented on both sides (left and right). The thorax hosts many muscles, and we selected
100 muscles with a function that can easily be determined and linked to particular tasks. First, muscles that
101 insert on the occiput and cervical apodemes are respectively direct and indirect head muscles that move
102 the head. Second, most leg muscles move the coxae but only one muscle per leg hosted inside the

103 thorax inserts on the second leg segment (the trochanter) and supports the body during locomotion.
104 Third, muscles that insert on the petiole are responsible for abdomen movement and support.

105 **2.3. Volume measurement**

106 We used ITK-SNAP to determine the volume of the nervous system, directly computed using the
107 count of labelled voxels and voxel size. However, this method would underestimate the actual volume
108 one muscle occupies in the thorax: the space observed between the fibres of a muscle is due to
109 shrinkage and needs to be included to determine physiological muscle volume. Therefore, we exported
110 mesh files (.stl) from ITK-SNAP to Blender 2.80. We fitted a 3D object - usually an Icosphere - to each
111 muscle mesh. Good fitting was achieved using 1) the 'Subsurface' modifier on the 3D object to create
112 more faces and 2) the 'Shrinkwrap' modifier to fit this 3D object on the muscle mesh. The volume of this
113 3D object was measured and used as the physiological muscle volume. Tendons were included in these
114 measurements, except the distal part of the external trochanter muscles, which enter the coxae. In case
115 of poor fitting, we split a muscle mesh into two meshes of simpler shapes and fitted a 3D object to both
116 separately. Volume values were calculated for both sides, added and then normalized using the volume
117 of the inner thorax, leading to proportions presented in figure 3 and table S2. The volume of the inner
118 thorax was determined by segmenting the whole thorax in a separate file., to which we subtracted the
119 volume of the exoskeleton (except the pro-, meso- and metafurcae, as they rise inside the thorax).
120 Soldiers and workers are two distinct morphological castes following different allometric rules, which is
121 why we used the whole thorax volume to scale our volume comparisons.

122 **2.4. Cuticle thickness measurement**

123 We measured cuticle thickness on 3D models using Drishti (Limaye, 2012). We created 3D
124 models directly from greyscale images (without segmentation) and inserted clipping planes through the
125 head (behind the eyes), pronotum (first dorsal thoracic plate) and the propodeum (first abdominal plate
126 but fused to the rest of the thorax in ants). The exact place of these clipping planes is shown in Figure
127 S1. Twelve measurements were taken for each individual along the left-right axis using the "Path"

128 function. Statistical analyses were performed using R 3.3.2 to compare worker and soldier cuticle
129 thickness normalized by head width values provided in Khalife and Peeters 2020. We tested data normal
130 distribution using Shapiro-Wilk's test and homoscedasticity using Bartlett's test. We applied Student's
131 two sample t-test when previous hypotheses were verified or Mann-Whitney's U test when not.

132

133 **3. Results**

134 **3.1. Thoracic nervous system (TNS)**

135 Hymenoptera have a ventral nervous system inside the thorax, consisting of three ganglia
136 connected by a nerve cord. In *C. perpusilla*, these ganglia are located near the insertion of the three leg
137 pairs (Figure 2). Ganglia and nerve cord occupy 19.1% of the thorax of the worker compared to 11.6%
138 for the soldier (Table S2). In terms of absolute volume, however, the TNS is larger in soldiers. The
139 allometry coefficient between the thoracic nervous system and the thorax - calculated as the ratio
140 between log (nervous system volume) and log (thorax volume) - is 0.87 for the soldier and 0.90 for the
141 worker. In addition, ganglia are located anteriorly to endoskeletal structures called furcae. The three
142 furcae are infoldings of the cuticle that rise like pillars inside the thorax anteriorly to each leg pair and
143 terminate with a platform where muscles attach. The first furca (or profurca) is particularly well-
144 developed to attach muscles that move the head (Figure 3). The profurca partly encloses the first
145 ganglion, hence a relatively bigger first ganglion in the worker implies a relatively higher profurca. Only a
146 tiny space is left between the profurca platform and the dorsal cuticle (pronotum), filled exclusively by
147 the oesophagus (Figure 2). In comparison, soldiers have more space between profurca and dorsal
148 cuticle, filled by the oesophagus but also muscle 46 (see Head muscles).

149 **3.2. Head muscles**

150 In *C. perpusilla*, the head is moved by five paired muscles (Figure 3, left). Three are direct and
151 insert on the occiput to move the head up and down while two are indirect and insert on cuticular

152 processes of the ventral plates (propleura) that push against the occiput to move the head sideways.

153 The muscles are as follow:

154 IvIm3: depressor of the head. Origin: anterior surface of the profurca (lateral), posterior margin
155 of the propleura. Insertion: ventrally on the occiput.

156 Itpm1+2: levator of the head. Origin: ventral, on the propleura. Insertion: dorsally on the
157 occiput. While other works clearly separate Itpm1 and Itpm2, we can hardly separate them in *C.*
158 *perpusilla* though we can clearly distinguish two tendons. We decided to group these two muscles as
159 Itpm1+2, similarly to muscle 42 in the honeybee (Snodgrass, 1956).

160 Idvm9: levator of the head. Origin: anterior surface of the profurca (mesiolateral). Insertion:
161 dorsally on the occiput.

162 Idvm5: levator/rotator of the propleura. Origin: broad, posterior 2/3 of the pronotum. Insertion:
163 cervical apodemes, anterodorsal prominence of the propleura.

164 IvIm1: stabilizer of the propleura. Origin: anterior surface of the profurca (mesial). Insertion:
165 cervical apodemes, anterodorsal prominence of the propleura.

166 We found relatively larger levator and depressor muscles in the worker (Figure 4). In contrast,
167 indirect muscles are much larger in the soldier (Figure 4). In particular, Idvm5 fills 14.1% of the thorax of
168 the soldier compared to 5.5% for the worker (see Table S2). For both castes, we determined head
169 volume related to the inner thorax volume. Values for head/thorax ratio were 1.23 for the worker and
170 1.05 for the soldier.

171 **3.3. External trochanter muscles**

172 Legs are moved by multiple muscles (eleven for the first leg pair). Most muscles inside the
173 thorax move the first leg segment ('coxa') and ensure its mobility in multiple directions for walking and
174 grooming. We focused on external trochanter (ET) muscles, originating inside the thorax and inserting
175 not on the first but on the second leg segment ('trochanter'). This is a singular case of muscles

176 connecting two non-consecutive segments, and they play a major role acting as traction cables holding
177 the body up during ground locomotion. There is one pair of ET muscles for each leg pair that are named
178 Iscm6, IIscm6 and IIIscm6 for the forelegs, midlegs and hindlegs, respectively.

179 Iscm6: depressor of the trochanter (forelegs). Origin: dorsal and posterior ridges of the
180 propleura. Insertion: proximal part of the trochanter.

181 IIscm6: depressor of the trochanter (midlegs). Origin: mesofurcal arms and mesonotum.
182 Insertion: proximal part of the trochanter.

183 IIIscm6: depressor of the trochanter (hindlegs). Origin: metapleural region (anterior). Insertion:
184 proximal part of the trochanter.

185 All ET muscles were relatively larger in the worker than in the soldier (Figure 4). In particular, the
186 relative volume of Iscm6 was 1.73 times higher in the worker, compared to 1.22 for IIscm6 and 1.28 for
187 IIIscm6 (Table S2).

188 **3.4. Petiole muscles**

189 Four muscles move the petiole by inserting directly on the top, bottom and sides of its anterior
190 end.

191 IA1: levator of the petiole. Origin: dorsally on the propodeum. Insertion: on a dorsal process of
192 the petiole.

193 IIIvlm2: depressor of the petiole. Origin: laterally on the propodeum. Insertion: mesioventrally
194 on the proximal part of the petiole. In other ants, this muscle has its origin on the metafurcal arms, but
195 the metafurca in *C. perpusilla* lacks arms.

196 IIIvlm3: torsion of the petiole. Origin: side of the metafurcal base. Insertion: laterally on the
197 proximal part of the petiole.

198 IA2: torsion of the petiole. Origin: dorsally on the propodeum, anterior to IA1. Insertion: laterally
199 on the proximal part of the petiole.

200 All four petiole muscles were relatively smaller in the worker (Figure 4). This difference was
201 subtle for small muscles IIIvIm2 and IIIvIm3 but more pronounced for large muscles IA1 and IA2 (Table
202 S2). In addition, we measured the ratio between abdomen volume and inner thorax for both castes.
203 Values were 1.52 for the worker and 2.75 for the soldier (replete).

204 3.5. Cuticle thickness

205 The head cuticle of soldiers was thicker than of workers ($11.4 \pm 0.7 \mu\text{m}$ compared to 5.9 ± 0.8
206 μm), even though this difference was not significant when normalized by head width (two sample t-test,
207 $t = -1.933$, $p=0.06998$, Figure 5). The soldier had a thinner thorax cuticle than head cuticle ($7.6 \pm 0.4 \mu\text{m}$
208 for the pronotum, $7.7 \pm 1.0 \mu\text{m}$ for the propodeum). In contrast, head and thorax had similar cuticle
209 thickness values for the worker ($5.4 \pm 0.6 \mu\text{m}$ for the pronotum, $6.2 \pm 1.0 \mu\text{m}$ for the propodeum). After
210 normalizing by head width, the thorax cuticle of the worker was thicker than of the soldier (pronotum:
211 Mann-Whitney U test, $U = 139.5$, $p<0.001$; propodeum: two sample t-test, $t = 5.7894$, $p<0.001$, Figure 5).
212 *Carebara perpusilla* workers and soldiers have moderate cuticle sculptures on the propodeum, which
213 explains the higher standard deviation we observed for this sclerite.

214

215 4. Discussion

216 4.1. Limited miniaturization of the nervous system

217 The TNS is relatively larger in minute workers than in soldiers: this result confirms the trend that
218 the central nervous system strongly increases as size decreases, particularly in Hymenoptera (Polilov
219 and Makarova, 2017). On a methodological point of view, our study complements the work of Lillico-
220 Ouachour et al. (2018), where the authors used microCT to look at the head internal anatomy of
221 workers and soldiers of *Pheidole*, another genus with minute workers. They showed that workers have a

222 relatively and even absolutely larger brain than soldiers. The brain-head allometric coefficients
223 computed from their data are 0.88 for soldiers and 0.97 for workers. In comparison, our results are less
224 spectacular: the relative (but not absolute) volume of the TNS increases in the worker, and we
225 calculated TNS-thorax allometric coefficients of 0.87 and 0.90 for the soldier and the worker,
226 respectively. However, the brain has many more functions than the TNS. Even though the numbers are
227 different, we still see an increase of the allometric coefficient in the worker, leading to similar
228 conclusions. In insects, the TNS is responsible for locomotion by instructing thoracic muscles. A smaller
229 brain limits the number and size of neurons (Makarova et al., 2021), Similarly, a smaller TNS likely
230 involves fewer and smaller neurons, which increases noise while reducing the space available for
231 mitochondria, resulting in a decrease in behavioral accuracy (reviewed by Niven and Farris, 2012). Ants
232 are well-known for their diversity of behavior compared to other miniaturised Hymenoptera, so the
233 nervous system (brain and TNS) is expected to be a major constraint on body size miniaturization in
234 ants. Interestingly, Haller's rule has been linked to behavioral repertoire in *Eciton* army ants (O'Donnell
235 et al., 2018). In this genus, large soldiers are specialized for colony defence against vertebrates whereas
236 polymorphic workers (smaller) forage, assemble the bivouac, and take care of the queen and brood.
237 Soldiers have a relatively smaller brain with a smaller sensory region, as a result of a colony-level
238 selection for minimal investment in brain tissue (O'Donnell et al., 2018). The size of the TNS thus seems
239 affected by two phenomena: miniaturization and functional constraints, linked to the behavioral
240 repertoire of each caste.

241 As a consequence of the limited reduction of TNS, the first ganglion of the worker is relatively
242 larger than of the soldier. Thus, the profurca rises relatively higher inside the thorax, leaving only a tiny
243 passage dorsally for the oesophagus. Haller's rule inside the thorax has downstream effects on the
244 entire thorax organisation.

245 **4.2. Head muscles are linked to division of labor**

246 For both soldiers and workers, we focused on three direct muscles (ltpm1+2, ldvm9 and lvm3)
247 in the prothorax. Larger ants previously studied have an additional direct muscle (ldlm1) that is missing
248 in *C. perpusilla* (Liu et al., 2019; Peeters et al., 2020). To investigate whether the lack of ldlm1 is specific
249 to subfamily Myrmicinae, we examined the thorax of larger myrmicines (*Messor barbarus* and
250 *Tetramorium fhg046*) and found ldlm1. In addition, it was also found in the minute workers of an Asian
251 *Carebara*, *C. castanea* (Khalife, personal data). The function of this muscle is to lift the head up, which is
252 redundant with ltpm1 and ldvm9 that can likely balance the loss of ldlm1 in *C. perpusilla* and possibly
253 other species with minute workers.

254 Even though workers and soldiers had the same direct and indirect head muscles, the relative
255 volumes of these muscles differed. Direct muscles – especially depressor muscles – occupied a slightly
256 larger proportion of the thorax in workers. In *C. perpusilla*, only workers carry loads: they can catch and
257 bring prey to the nest, but also move soil pellets to bury insect carcasses they feed on (Khalife and
258 Peeters, 2020). Direct muscles are responsible for the up and down movement of the head and are likely
259 involved in these tasks. Remarkably, the head/inner thorax volume ratio is higher for workers than
260 soldiers despite the apparently large head of soldiers, explaining why workers require relatively larger
261 direct head muscles. Moreover, soldiers do not carry anything but their head, hence direct muscles are
262 only required for unloaded up and down movements. Conversely, indirect head muscles are much
263 bigger in soldiers. This major difference is explained by ldvm5 that fills most of the soldier prothorax. In
264 *C. perpusilla*, older soldiers either guard the nest entrance or bite through the cuticle of insect carcasses
265 to create holes so that workers can reach inside (Khalife and Peeters, 2020). Their large head is packed
266 with powerful mandible muscles and needs to be manoeuvred sideways efficiently for fighting and
267 biting. Division of labor can thus explain differences in head muscle allocation between workers and
268 soldiers.

269 **4.3. Leg muscles and body balance**

270 All three ET muscles are relatively larger in workers than in soldiers. Since only workers carry
271 objects, loaded locomotion requires strong ET muscles to support the combined weight of the worker
272 and its load across all legs. However, some leg pairs have specific functions that we can discuss in more
273 details. First, *C. perpusilla* workers carry their loads with their mandibles, which likely shifts their centre
274 of mass forward in a similar way as harvester ants (Merienne et al., 2020): strong Iscm6 are required to
275 support a greater weight with the forelegs. Second, soldiers have a huge head packed with mandible
276 muscles, and a large majority soldiers stay inside the nest and store food inside their huge abdomen,
277 functioning as repletes (Khalife and Peeters 2020). Contrary to loaded workers, replete soldiers are well
278 balanced along the anteroposterior axis as their head and abdomen are heavy. We believe that their
279 center of mass is perfectly set in the thorax, resulting from a stabilizing selection modelled by Anderson
280 et al. (2020). In addition, replete soldiers stay in the nest, do not move much and can rest their head and
281 abdomen on the floor, meaning that ET muscles often carry a relatively low weight.

282 **4.4. Petiole muscles for abdomen support**

283 All four petiole muscles are relatively larger in the soldier than in the worker. Petiole muscles
284 are responsible for moving the abdomen: petiole and post-petiole are small segments compared to the
285 posterior thorax (propodeum) so the muscles they house are extremely small in comparison. Neither
286 workers nor soldiers of *C. perpusilla* bend their abdomen to sting or spray acid. However, soldiers can
287 store food in their crop up to 92% of the abdomen volume (Khalife and Peeters, 2020). Replete soldiers'
288 abdomen is 2.75 times bigger than the inner thorax, hence large petiole muscles are required to support
289 and lift off the ground a replete abdomen. In comparison, workers store food in their abdomen more
290 moderately and their abdomen is only 1.52 times bigger than their inner thorax, so they do not require
291 large petiole muscles.

292 **4.5. The basis of strength in minute ants**

293 The square-cube law is commonly used to explain the strength of minute ants. A smaller insect
294 has a greater area to volume ratio, so muscles are relatively stronger. Our results show that in addition

295 to physics, the space allocated to muscles is crucial. Division of labor implies that workers do not need to
296 disperse or reproduce, which allows a trade-off in ant workers (Peeters et al., 2020): by investing less
297 energy and space in flight and reproductive apparatuses but more in other muscles, they retain a
298 powerful muscle set even in a miniaturized form. In contrast, solitary Hymenoptera function as
299 autonomous units that need to fly and reproduce, hence they cannot display the same physical abilities
300 as ant workers while miniaturizing. However, the smallest ants remain longer than a millimeter, which is
301 still larger than the smallest Hymenoptera (*e.g.* 250 μ m, see Polilov et al., 2015 for examples). Other
302 selective pressures likely limit the miniaturization of workers, and are still to be discovered.

303 **4.6. Cuticle thickness: miniaturization and colonial economy**

304 After normalisation by head width, workers had a thicker thorax cuticle than soldiers whereas
305 no significant difference was observed for the head cuticle. This observation suggests that the
306 exoskeleton reduces allometrically, contrary to what is usually observed in insects (Polilov and
307 Makarova, 2017), meaning that thorax cuticle could limit miniaturization in ants. The smallest insects
308 have a simplified exoskeleton with a reduction of number of segmented structures (Polilov, 2015). Here
309 *C. perpuzilla* workers have a sclerotized exoskeleton with no missing sclerite. The exoskeleton of ants is
310 a scaffold where many muscles find their insertion and would likely lose some crucial biomechanical
311 properties if simplified. In addition, the small difference in absolute cuticle thickness of the thorax
312 between workers and soldiers minimizes the extra production cost of soldiers. This result contrasts with
313 data in *Carebara diversa* where minute workers (head width 0.55-0.61 mm) with a cuticle thickness
314 between 5.4 and 8.4 μ m coexist with soldiers (head width 4.27-4.74 mm) with a cuticle thickness
315 between 37.6 and 50.6 μ m (Peeters et al., 2017a). In *C. perpuzilla* worker/soldier dimorphism is less
316 pronounced, thorax cuticle thickness is similar, and more soldiers can be produced at the colony scale.
317 However, the head cuticle was thicker than the thorax cuticle in soldiers. Even though it increases the
318 production cost of soldiers, thicker head cuticle confers robustness to guard the nest against invaders
319 and attach big mandible muscles.

320 **4.7. Conclusion**

321 The central nervous system (brain and thoracic ganglia) appears to be the principal constraint on
322 miniaturization in ants (Niven and Farris, 2012; Polilov, 2015; Polilov and Makarova, 2017). Our results
323 show intraspecific differences in how this affects space allocation inside the thorax between minute
324 workers and larger soldiers. Because the first thoracic ganglion remains large, the profurca must be
325 large enough to encircle it, which influences the organisation of all head muscles. Miniaturization
326 requires trade-offs: with less space available, some muscles are reduced whereas others remain large. In
327 *C. perpusilla*, workers have reduced petiole muscles but substantial nervous system and leg muscles.
328 However, these trade-offs may be linked to the behavioral repertoire of minute ants, which can vary
329 between species. The thorax includes many other important structures such as the oesophagus and
330 glands, and a full assessment of how these structures change with body size, and how the thorax design
331 in general varies with ecology and behavior, remains for future work.

332

333 **Author statement**

334 Adam Khalife: Investigation, Visualization, Writing – Original Draft.

335 Christian Peeters: Supervision, Conceptualization, Resources, Validation, Writing – Review and Editing.

336 Evan Economo: Supervision, Conceptualization, Resources, Writing – Review and Editing.

337

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344

345 **Declarations of interest**

346 The authors declare they have no competing interests.

347

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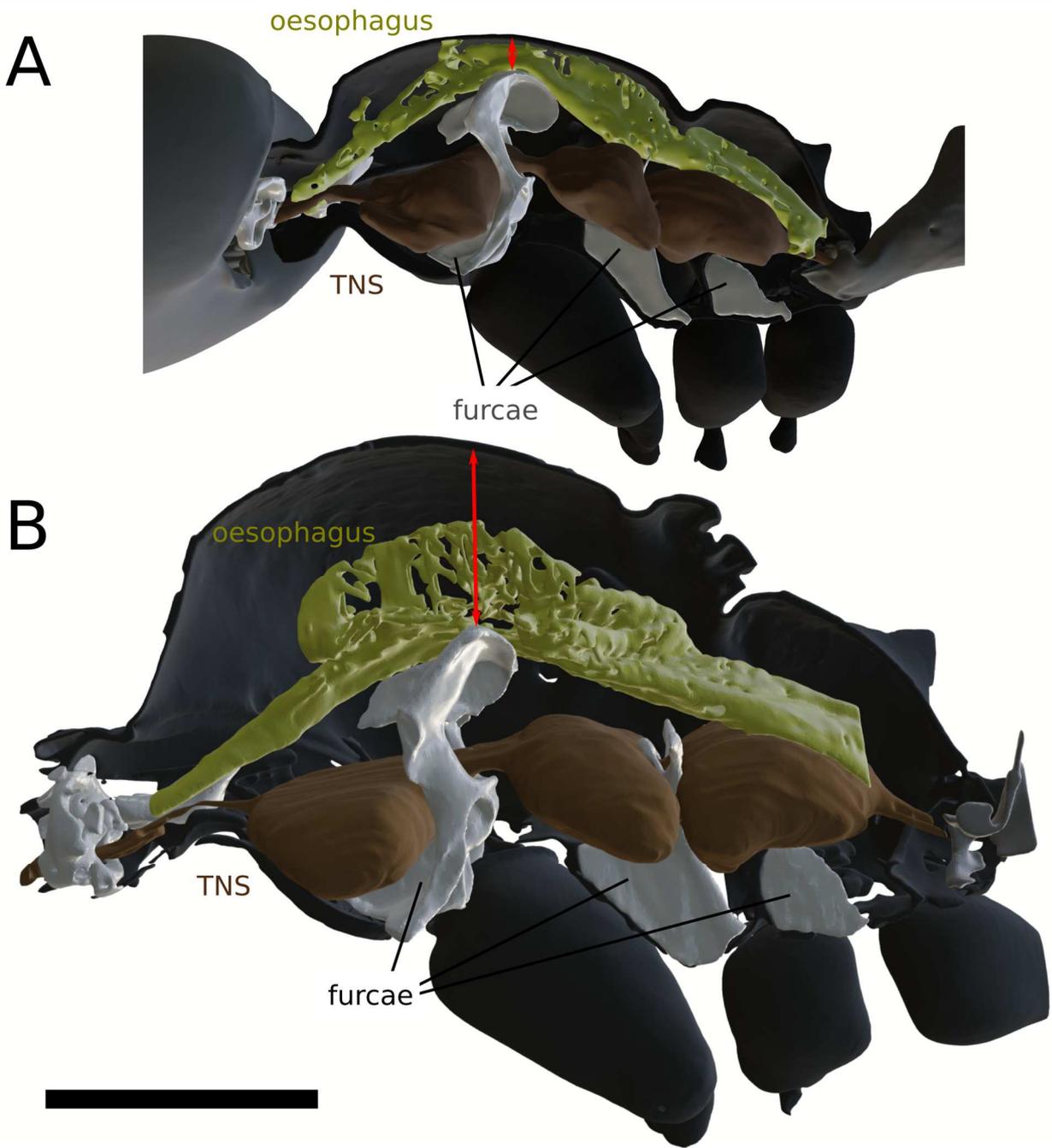
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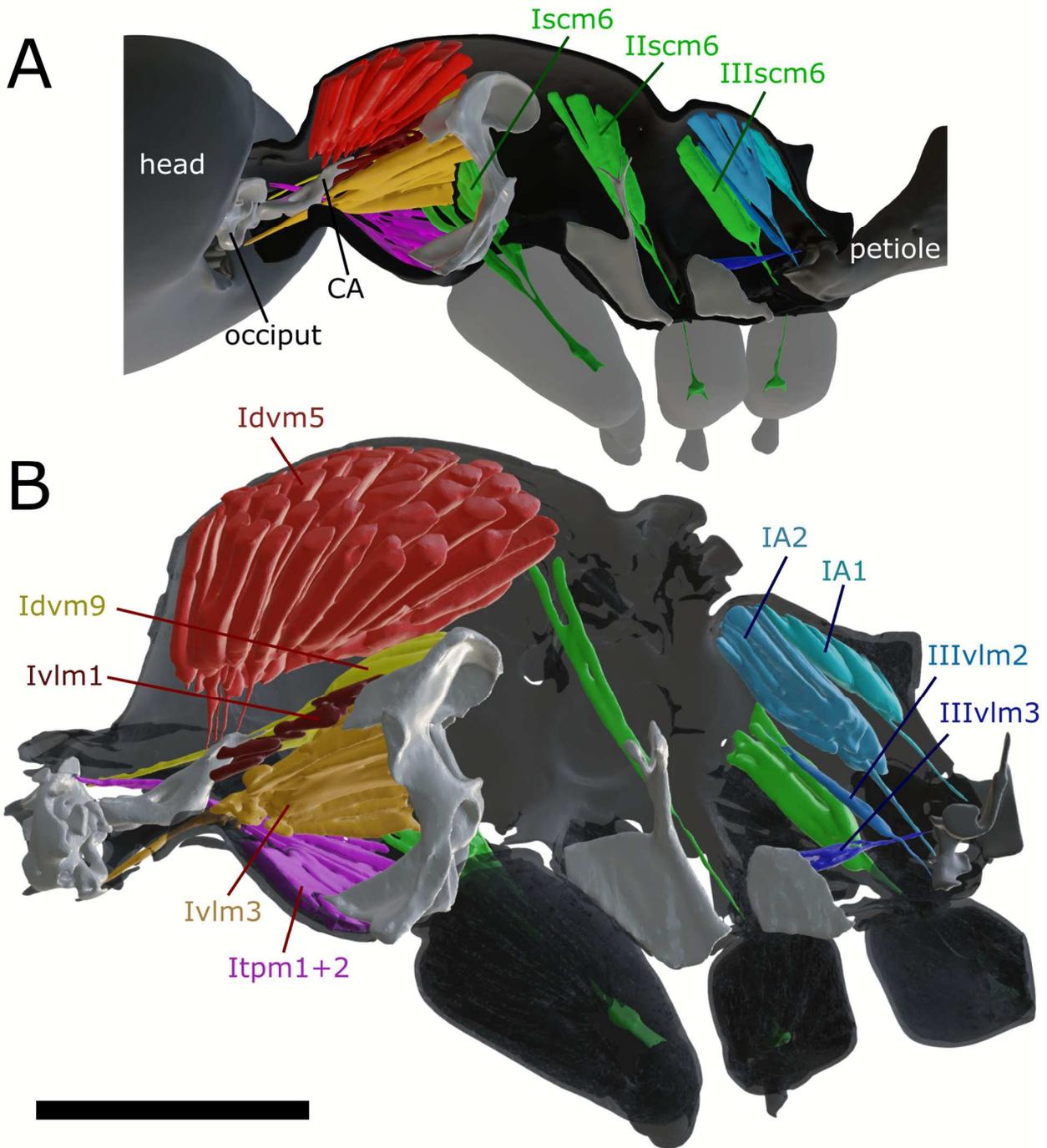


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408 **Figure 1:** Photograph of a *C. perpusilla* colony fragment showing workers, soldiers and brood.



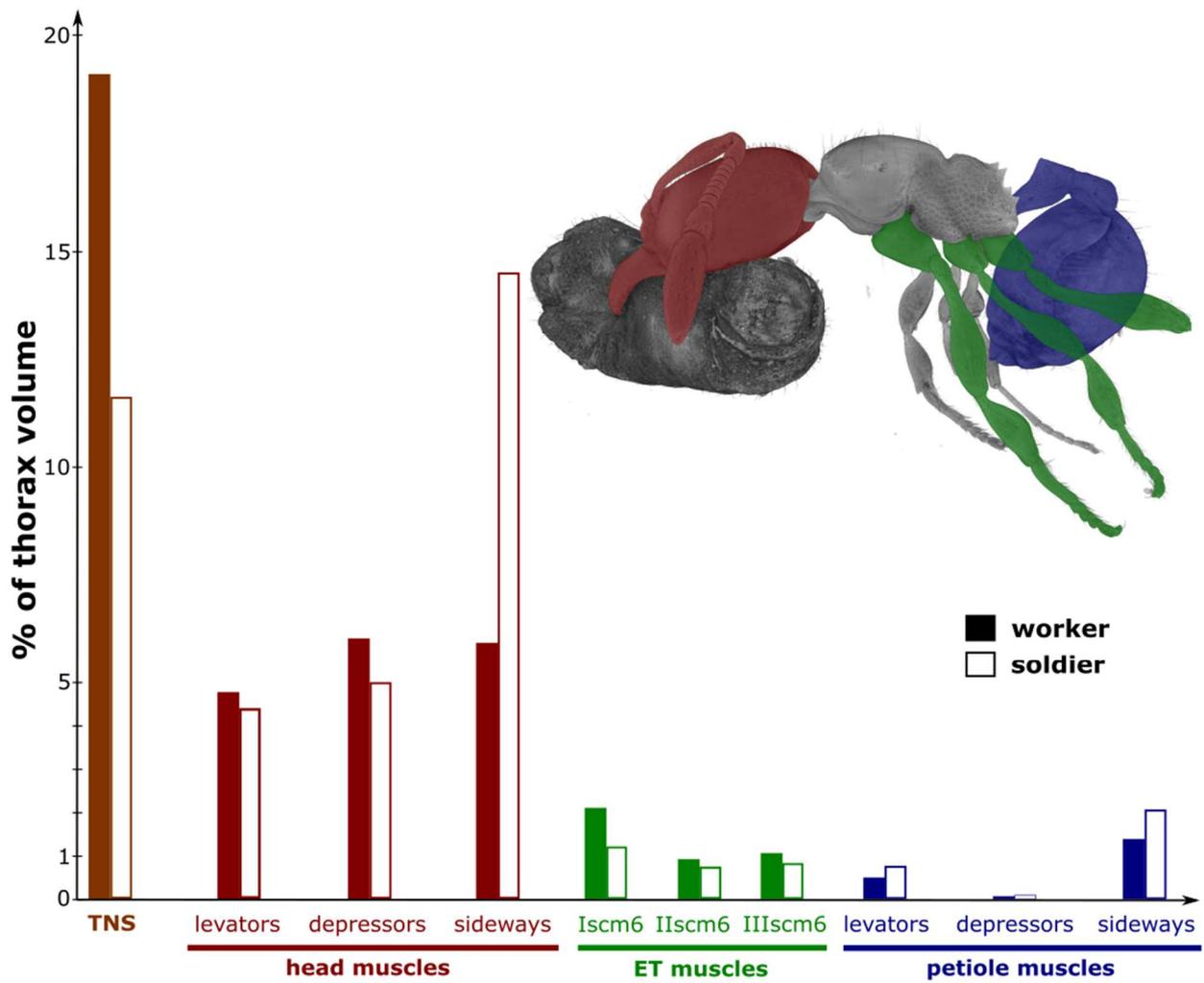
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410 **Figure 2:** 3D reconstruction of the furcae (light grey), oesophagus (green) and neural ganglia (brown) of *C.*
 411 *perpusilla* worker (A) and soldier (B), same scale. Furcae are part of the endoskeleton, and the profurca (first
 412 segment) almost touches the thorax roof in the worker, leaving only a tiny gap (red double arrow) for the
 413 oesophagus. TNS: thoracic nervous system; scale bar = 200 μm . The oesophagus was segmented automatically for
 414 visualization purposes only, hence we did not perform slice-by-slice manual correction, which explains its strange
 415 aspect.



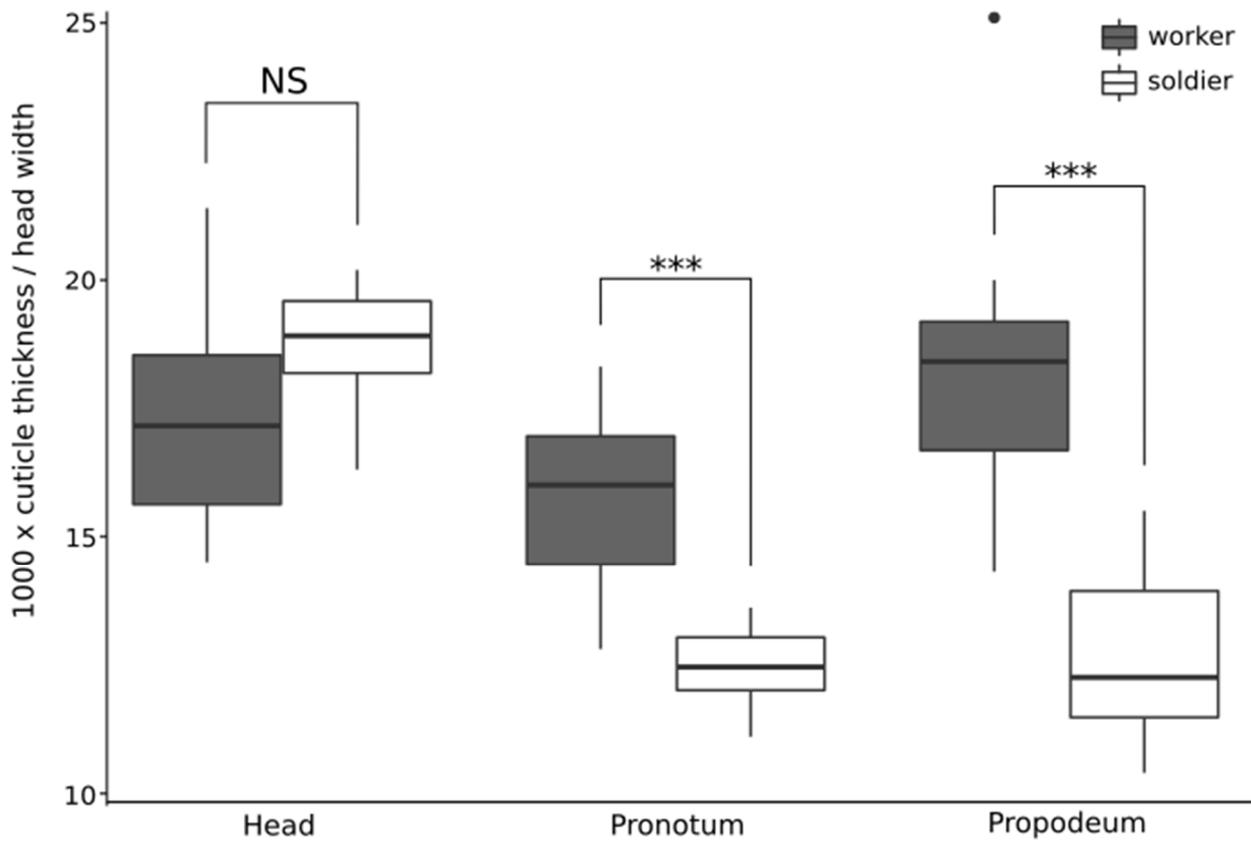
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417 **Figure 3:** 3D reconstruction of head, external trochanter and petiole muscles in *C. perpusilla* worker (A) and soldier
 418 (B), same scale. Structures and muscles are annotated either on worker or soldier for clarity. Muscle names follow
 419 the nomenclature of Aibekova et al. (2022). CA: cervical apodemes; scale bar = 200 μ m.



420

421 **Figure 4:** Comparison of the relative volume of neural ganglia and muscles inside the thorax between worker and
 422 soldier of *C. perpusilla*. Muscles were grouped according to their function, determined from their insertion site.
 423 TNS: thoracic nervous system; ET: external trochanter.



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Figure 5: Boxplot comparing worker (grey) and soldier (white) cuticle thickness normalized by head width. Medians, quartiles and ranges are displayed. NS: non-significant difference. ***: $p < 0.001$.