Minute Workers and Large Soldiers in the Subterranean Ant Carebara Perpusilla: Musculoskeletal Consequences of Haller's Rule in the Thorax

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

Adam Khalife[1,2,*], Christian Peeters[2*] and Evan Economo[3,4]

[1]Laboratoire Mer-Molécules-Santé (MMS-BiOSSE), Le Mans Université, 72000 Le Mans, France

[2]Institute of Ecology and Environmental Sciences (iEES-Paris), Sorbonne Université, 75005 Paris, France

[3]Biodiversity and Biocomplexity Unit, Okinawa Institute of Science and Technology Graduate University (OIST), Okinawa, Japan

[4]Radcliffe Institute for Advanced Study, Harvard University, Cambridge, MA, 02445, USA

*Corresponding author: ak.linkario@gmail.com

Abstract

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

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

1. Introduction

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

Miniaturization is the evolution of extremely small body size within a lineage (Hanken and Wake, 1993). This phenomenon is widespread in insects and has driven the evolution of the smallest metazoans (parasitoid wasps, see Polilov, 2015 for examples) as well as the evolution of ants. Indeed, flight loss alleviated constraints on body size in worker ants, thus permitting miniaturization in most genera, contrary to social bees and wasps (Peeters and Ito, 2015). During 140 Mya of evolution, relatively few species of ants became large, e.g. in *Dinoponera*, 30mm, 8 spp (Lenhart et al., 2013), *Paraponera*, 25mm, 1 sp (AntWeb, 2022), and *Dinomyrmex*, 28mm, 2 spp (AntWeb, 2022). In contrast, workers became minute – body size below 2mm – in most extant species (Peeters and Ito, 2015), e.g. in Myrmicinae genera *Monomorium*, 400 spp, *Carebara*, 250 spp, and *Pheidole*, 1100 spp (measured from AntWeb pictures, AntWeb, 2022). Miniaturization brings many benefits to ants, such as opening up new interstitial niches and reducing the cost and development time of individuals (Peeters and Ito, 2015). However, miniaturization is more than a simple downscaling of individuals as the size of some organs changes allometrically with body size. A famous example is Haller's rule brain-body allometry, defined as the brain getting relatively bigger with decreasing body size (Rensch, 1948). This rule has been validated empirically in ants (Cole, 1985; Seid et al., 2011) as well as many vertebrate and invertebrate taxa (reviewed by Eberhard and Wcislo, 2011). Moreover, miniaturization involves a reduction of sensory organs like the eyes, and smaller ants - with less ommatidia - have a lower spatial resolution and contrast sensitivity (Palavalli-Nettimi and Narendra, 2018; Palavalli-Nettimi et al., 2019). Nonetheless, how vital organs and muscles scale with miniaturization inside the thorax is unknown. The internal morphology of the thorax has only been studied in large ants that are not representative of the majority of ants (Liu et al., 2019; Peeters et al., 2020; Aibekova et al., 2022). The strength of small ants is commonly explained with physics and the square-cube law: the volume of the thorax is a cube measurement (x^3) whereas muscle strength is proportional to its cross-sectional area, a square measurement (x^2). The ratio between muscle strength and thorax volume ($x^2/x^3 = 1/x$) increases when size (x) decreases, explaining why smaller animals can carry relatively heavier loads in general. However, ants stand out in terms of load carrying, and a minute ant thorax must integrate functional organs (*e.g.* from the nervous and digestive systems) along with numerous muscles in a tiny volume in order to complete worker tasks.

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

2. Material and Methods

2.1. Specimens and scanning

Specimens of *C. perpusilla* were collected in a riverine forest on August 2016 in Gorongosa National Park, Sofala province, Mozambique (S 19.00133 E 34.37730, ~ 100m elevation) and stored in 90% ethanol. Micro-CT scans were performed at the Okinawa Institute of Science and Technology Graduate University (OIST, Japan) using a Zeiss Xradia 510 Versa 3D X-ray microscope operated with the Zeiss Scout-and-Scan Control System software (version 11.1.6411.17883). One worker and one soldier, selected randomly, were stained in a 2 M I_2E solution for 24 h (iodine dissolved in 99% ethanol, see Gignac et al., 2016), and then transferred into microtubes filled with 99% ethanol for scanning. Scan settings were selected to yield optimum scan quality. Full 360-degree rotations were based on 1601 projections. The resulting scan resolutions and parameter settings are provided in Table S1. Post-imaging 3D reconstruction was done with the Zeiss Scout-and-Scan Control System Reconstructor software (version 11.1.6411.17883), and the output files saved in DICOM format. While we performed 3D quantitative analyses on just one specimen per caste, we validated our observations with thorax dissections and histological sections of other individuals.

2.2. Segmentation

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

2.3. Volume measurement

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

2.4. Cuticle thickness measurement

We measured cuticle thickness on 3D models using Drishti (Limaye, 2012). We created 3D models directly from greyscale images (without segmentation) and inserted clipping planes through the head (behind the eyes), pronotum (first dorsal thoracic plate) and the propodeum (first abdominal plate but fused to the rest of the thorax in ants). The exact place of these clipping planes is shown in Figure S1. Twelve measurements were taken for each individual along the left-right axis using the "Path" function. Statistical analyses were performed using R 3.3.2 to compare worker and soldier cuticle thickness normalized by head width values provided in Khalife and Peeters 2020. We tested data normal distribution using Shapiro-Wilk's test and homoscedasticity using Bartlett's test. We applied Student's two sample t-test when previous hypotheses were verified or Mann-Whitney's U test when not.

3. Results

3.1. Thoracic nervous system (TNS)

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

3.2. Head muscles

In *C. perpusilla*, the head is moved by five paired muscles (Figure 3, left). Three are direct and insert on the occiput to move the head up and down while two are indirect and insert on cuticular processes of the ventral plates (propleura) that push against the occiput to move the head sideways. The muscles are as follow:

Ivlm3: depressor of the head. Origin: anterior surface of the profurca (lateral), posterior margin of the propleura. Insertion: ventrally on the occiput.

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

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

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

Ivlm1: stabilizer of the propleura. Origin: anterior surface of the profurca (mesial). Insertion: cervical apodemes, anterodorsal prominence of the propleura.

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

3.3. External trochanter muscles

Legs are moved by multiple muscles (eleven for the first leg pair). Most muscles inside the thorax move the first leg segment ('coxa’) and ensure its mobility in multiple directions for walking and grooming. We focused on external trochanter (ET) muscles, originating inside the thorax and inserting not on the first but on the second leg segment ('trochanter'). This is a singular case of muscles connecting two non-consecutive segments, and they play a major role acting as traction cables holding the body up during ground locomotion. There is one pair of ET muscles for each leg pair that are named Iscm6, IIscm6 and IIIscm6 for the forelegs, midlegs and hindlegs, respectively.

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

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

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

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

3.4. Petiole muscles

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

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

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

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

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

All four petiole muscles were relatively smaller in the worker (Figure 4). This difference was subtle for small muscles IIIvlm2 and IIIvlm3 but more pronounced for large muscles IA1 and IA2 (Table S2). In addition, we measured the ratio between abdomen volume and inner thorax for both castes. Values were 1.52 for the worker and 2.75 for the soldier (replete).

3.5. Cuticle thickness

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

4. Discussion

4.1. Limited miniaturization of the nervous system

The TNS is relatively larger in minute workers than in soldiers: this result confirms the trend that the central nervous system strongly increases as size decreases, particularly in Hymenoptera (Polilov and Makarova, 2017). On a methodological point of view, our study complements the work of Lillico-Ouachour et al. (2018), where the authors used microCT to look at the head internal anatomy of workers and soldiers of *Pheidole*, another genus with minute workers. They showed that workers have a relatively and even absolutely larger brain than soldiers. The brain-head allometric coefficients computed from their data are 0.88 for soldiers and 0.97 for workers. In comparison, our results are less spectacular: the relative (but not absolute) volume of the TNS increases in the worker, and we calculated TNS-thorax allometric coefficients of 0.87 and 0.90 for the soldier and the worker, respectively. However, the brain has many more functions than the TNS. Even though the numbers are different, we still see an increase of the allometric coefficient in the worker, leading to similar conclusions. In insects, the TNS is responsible for locomotion by instructing thoracic muscles. A smaller brain limits the number and size of neurons (Makarova et al., 2021), Similarly, a smaller TNS likely involves fewer and smaller neurons, which increases noise while reducing the space available for mitochondria, resulting in a decrease in behavioral accuracy (reviewed by Niven and Farris, 2012). Ants are well-known for their diversity of behavior compared to other miniaturised Hymenoptera, so the nervous system (brain and TNS) is expected to be a major constraint on body size miniaturization in ants. Interestingly, Haller's rule has been linked to behavioral repertoire in *Eciton* army ants (O'Donnell et al., 2018). In this genus, large soldiers are specialized for colony defence against vertebrates whereas polymorphic workers (smaller) forage, assemble the bivouac, and take care of the queen and brood. Soldiers have a relatively smaller brain with a smaller sensory region, as a result of a colony-level selection for minimal investment in brain tissue (O'Donnell et al., 2018). The size of the TNS thus seems affected by two phenomena: miniaturization and functional constraints, linked to the behavioral repertoire of each caste.

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

4.2. Head muscles are linked to division of labor

For both soldiers and workers, we focused on three direct muscles (Itpm1+2, Idvm9 and Ivlm3) in the prothorax. Larger ants previously studied have an additional direct muscle (Idlm1) that is missing in *C. perpusilla* (Liu et al., 2019; Peeters et al., 2020). To investigate whether the lack of Idlm1 is specific to subfamily Myrmicinae, we examined the thorax of larger myrmicines (*Messor barbarus* and *Tetramorium fhg046*) and found Idlm1. In addition, it was also found in the minute workers of an Asian *Carebara*, *C. castanea* (Khalife, personal data). The function of this muscle is to lift the head up, which is redundant with Itpm1 and Idvm9 that can likely balance the loss of Idlm1 in *C. perpusilla* and possibly other species with minute workers.

Even though workers and soldiers had the same direct and indirect head muscles, the relative volumes of these muscles differed. Direct muscles – especially depressor muscles – occupied a slightly larger proportion of the thorax in workers. In *C. perpusilla*, only workers carry loads: they can catch and bring prey to the nest, but also move soil pellets to bury insect carcasses they feed on (Khalife and Peeters, 2020). Direct muscles are responsible for the up and down movement of the head and are likely involved in these tasks. Remarkably, the head/inner thorax volume ratio is higher for workers than soldiers despite the apparently large head of soldiers, explaining why workers require relatively larger direct head muscles. Moreover, soldiers do not carry anything but their head, hence direct muscles are only required for unloaded up and down movements. Conversely, indirect head muscles are much bigger in soldiers. This major difference is explained by Idvm5 that fills most of the soldier prothorax. In *C. perpusilla*, older soldiers either guard the nest entrance or bite through the cuticle of insect carcasses to create holes so that workers can reach inside (Khalife and Peeters, 2020). Their large head is packed with powerful mandible muscles and needs to be manoeuvred sideways efficiently for fighting and biting. Division of labor can thus explain differences in head muscle allocation between workers and soldiers.

4.3. Leg muscles and body balance

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

4.4. Petiole muscles for abdomen support

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

4.5. The basis of strength in minute ants

The square-cube law is commonly used to explain the strength of minute ants. A smaller insect has a greater area to volume ratio, so muscles are relatively stronger. Our results show that in addition to physics, the space allocated to muscles is crucial. Division of labor implies that workers do not need to disperse or reproduce, which allows a trade-off in ant workers (Peeters et al., 2020): by investing less energy and space in flight and reproductive apparatuses but more in other muscles, they retain a powerful muscle set even in a miniaturized form. In contrast, solitary Hymenoptera function as autonomous units that need to fly and reproduce, hence they cannot display the same physical abilities as ant workers while miniaturizing. However, the smallest ants remain longer than a millimeter, which is still larger than the smallest Hymenoptera (*e.g.* 250µm, see Polilov et al., 2015 for examples). Other selective pressures likely limit the miniaturization of workers, and are still to be discovered.

4.6. Cuticle thickness: miniaturization and colonial economy

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

4.7. Conclusion

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

Author statement

Adam Khalife: Investigation, Visualization, Writing – Original Draft.
Christian Peeters: Supervision, Conceptualization, Resources, Validation, Writing – Review and Editing.
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Declarations of interest

The authors declare they have no competing interests.

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Figures

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

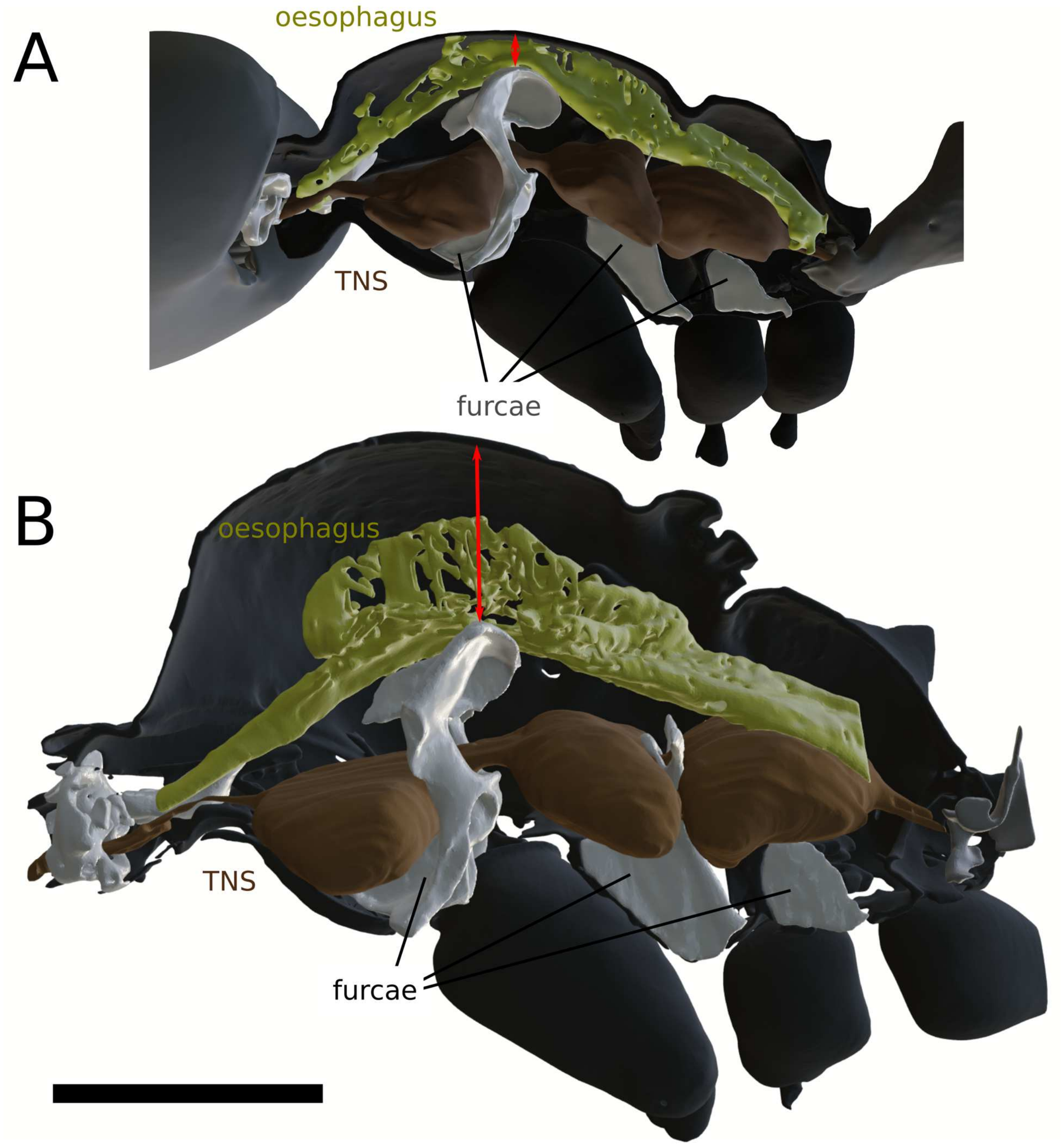

Figure 2: 3D reconstruction of the furcae (light grey), oesophagus (green) and neural ganglia (brown) of *C. perpusilla* worker (A) and soldier (B), same scale. Furcae are part of the endoskeleton, and the profurca (first segment) almost touches the thorax roof in the worker, leaving only a tiny gap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

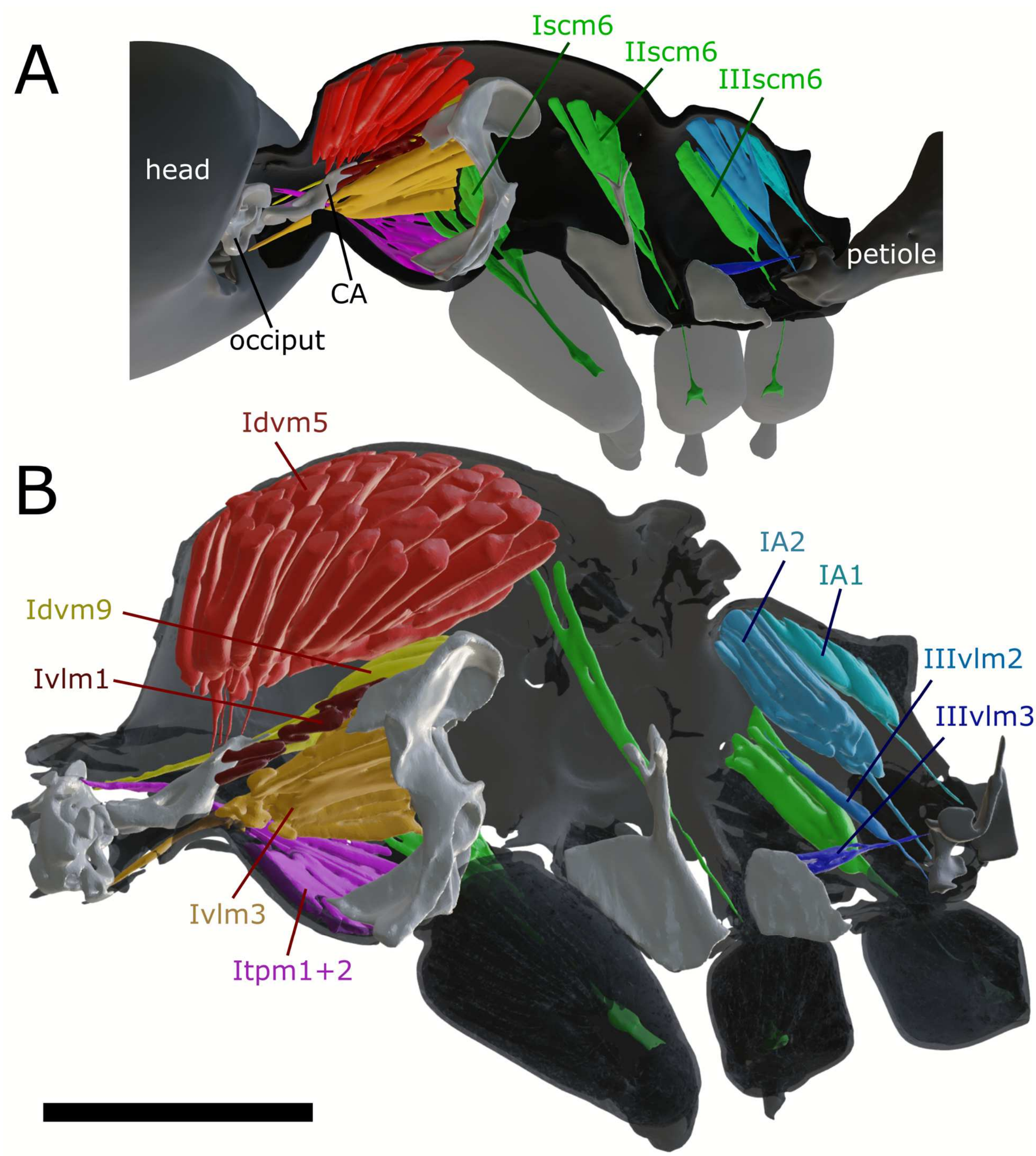

Figure 3: 3D reconstruction of head, external trochanter and petiole muscles in *C. perpusilla* worker (A) and soldier (B), same scale. Structures and muscles are annotated either on worker or soldier for clarity. Muscle names follow the nomenclature of Aibekova et al. (2022). CA: cervical apodemes;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

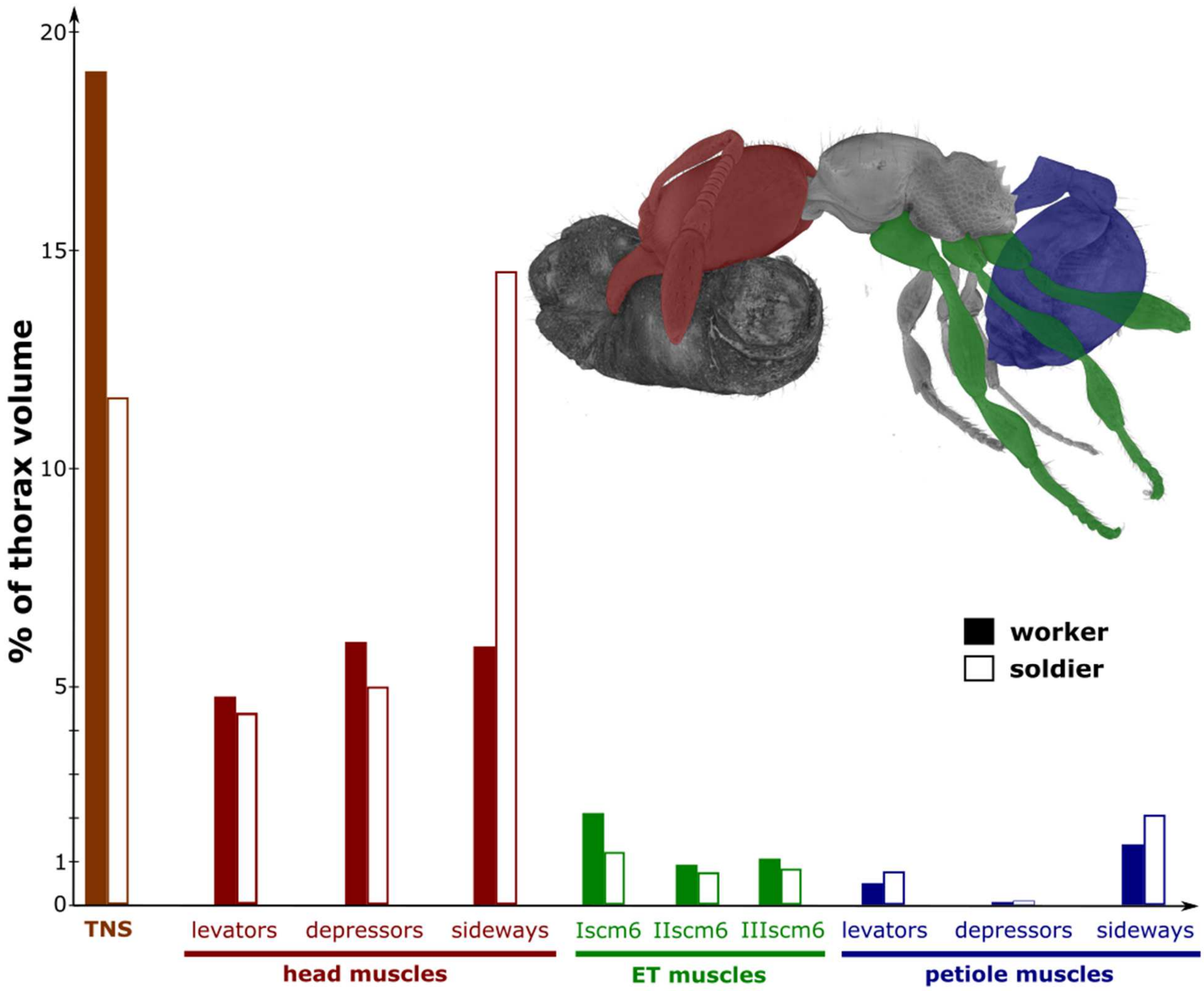

Figure 4: Comparison of the relative volume of neural ganglia and muscles inside the thorax between worker and soldier of *C. perpusilla*. Muscles were grouped according to their function, determined from their insertion site. TNS: thoracic nervous system; ET: external trochanter.

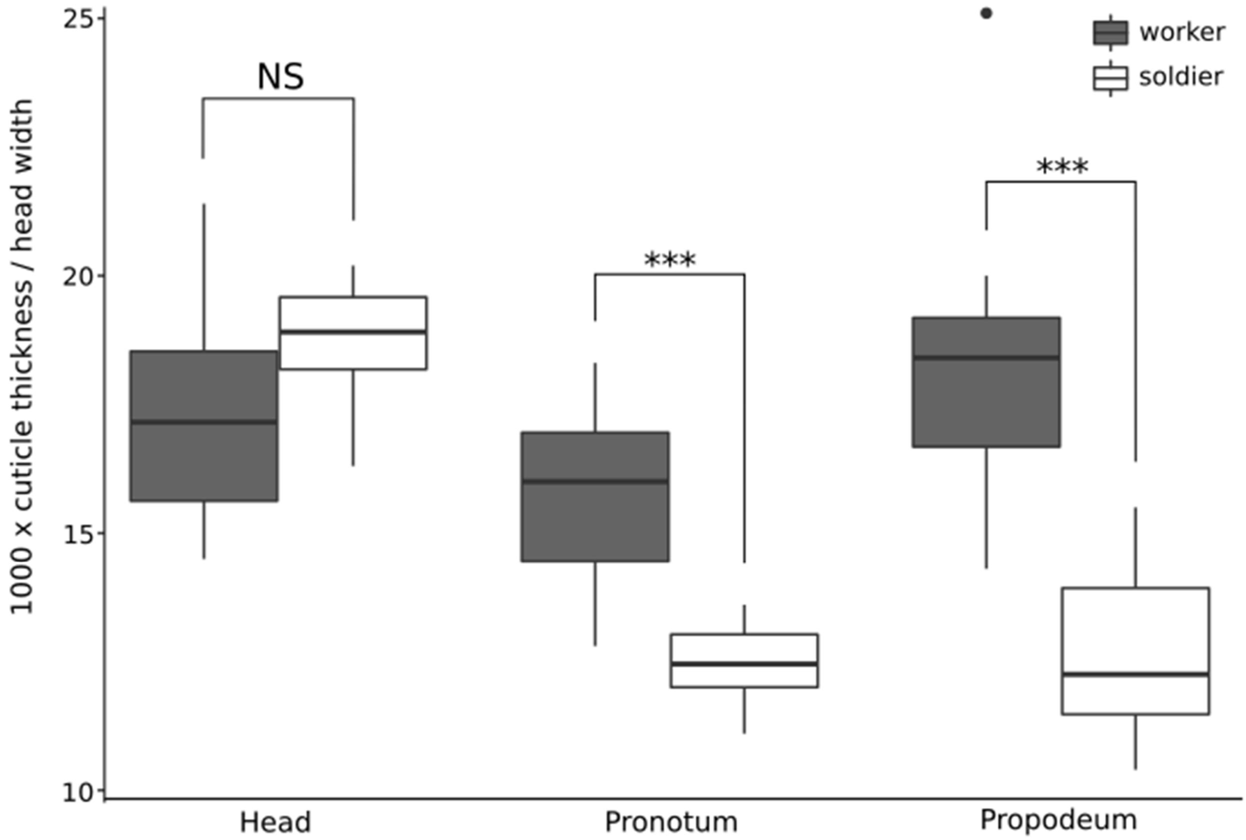

Figure 5: Boxplot comparing worker (grey) and soldier (white) cuticle thickness normalized by head width. Medians, quartiles and ranges are displayed. NS: non-significant difference. ***: p<0.001.