

# Internal head morphology of minor workers and soldiers in the hyperdiverse ant genus *Pheidole*<sup>1</sup>

Angelica Lillico-Ouachour, Brian Metscher, Tominari Kaji, and Ehab Abouheif

**Abstract:** In the hyperdiverse ant genus *Pheidole* Westwood, 1839, the worker caste evolved into two morphologically distinct subcastes: minor workers and soldiers. The evolution of soldiers, which are larger in size than minor workers and have disproportionately larger heads, are thought to be key to *Pheidole*'s success. Although many studies have focused on external anatomy, little is known about their internal anatomy. We therefore used microCT imaging and quantitative three-dimensional image analysis to reconstruct the major glands of the head, the musculature, nervous system, and digestive organ of minor workers and soldiers of four *Pheidole* species. We expected these tissues to scale isometrically and to be proportionally larger in soldiers relative to the minor workers. Surprisingly, we found that the nervous system, cephalic gland, and digestive organ volume are absolutely and relatively smaller in soldiers, whereas muscle volume is absolutely and relatively larger, than in minor workers. This may reflect individual-level trade-offs, where muscles grow at the expense of all other cephalic organs. Alternatively, this relationship may reflect the specialization of internal anatomy in each subcaste to enhance division of labour at the colony level. Future studies should test these alternative hypotheses across a larger number of *Pheidole* species.

**Key words:** *Pheidole*, ants, soldiers, ecoevodevo, glands, polyphenism, microCT, internal anatomy, developmental plasticity, allometry, brain volume, muscle.

**Résumé :** Dans le genre de fourmis *Pheidole* Westwood, 1839 caractérisé par une hyperdiversité, l'évolution de la caste des ouvrières a mené à deux sous-castes distinctes sur le plan morphologique, soit les ouvrières mineures et les soldates. L'apparition des soldates, qui sont de plus grande taille que les ouvrières mineures et ont des têtes disproportionnellement grandes, serait un aspect clé du succès des *Pheidole*. Si de nombreuses études se sont penchées sur leur anatomie externe, les connaissances sur leur anatomie interne sont très limitées. Nous avons donc utilisé la microtomographie par ordinateur (microCT) et l'analyse quantitative d'images tridimensionnelles pour reconstituer les principales glandes de la tête, la musculature, le système nerveux et l'organe digestif d'ouvrières mineures et de soldates de quatre espèces de *Pheidole*. Nous nous attendions à ce que la taille de ces tissus varie de manière isométrique et qu'elle soit proportionnellement plus grande chez les soldates que chez les ouvrières mineures. Étonnamment, nous avons constaté que, sur une base tant absolue que relative, le volume du système nerveux, des glandes céphaliques et de l'organe digestif est plus petit, alors que le volume des muscles est plus grand chez les soldates que chez les ouvrières mineures. Ces relations pourraient refléter des compromis au niveau individuel qui font que les muscles croissent au détriment de tous les autres organes céphaliques ou encore une spécialisation de l'anatomie interne des différentes sous-castes pour rehausser la division du travail au niveau de la colonie. Des études futures devraient vérifier ces différentes hypothèses pour un grand nombre d'espèces de *Pheidole*. [Traduit par la Rédaction]

**Mots-clés :** *Pheidole*, fourmis, soldates, écoevodévo, glandes, polyphénisme, microCT, anatomie interne, plasticité développementale, allométrie, volume du cerveau, muscle.

## Introduction

The scaling relationship or allometry between body parts accounts for a large fraction of the phenotypic diversity in ants, both within and among species (Huxley 1932; Wilson 1953; Gould 1966; Lande 1979; Pie and Traniello 2007; Shingleton et al. 2007; Economo et al. 2015; Mirth et al. 2016). Every ant colony has a reproductive division of labour that consists of the queen caste, primarily responsible for reproduction, and the worker caste, primarily responsible for all other tasks in the colony (Hölldobler and Wilson 1990, 2009). In addition to a reproductive division of

labour, at least 15 genera have independently evolved morphologically distinct subcastes within the worker caste (Wilson 1979, 1980, 1983, 1984, 2003; Hölldobler and Wilson 1990; Blanchard and Moreau 2017). These physical worker subcastes are characterized by size and allometric differences between head and body size, which allow workers to more effectively perform specific tasks in the colony (Wilson 1979, 1984, 1985b; Hölldobler and Wilson 1990; Schmid-Hempel 1992; Yang et al. 2004; Pie and Traniello 2007).

In the hyperdiverse ant genus *Pheidole* Westwood, 1839, the worker caste comprises at least two subcastes: minor workers and soldiers (also known as major workers) (Hölldobler and Wilson

Received 24 July 2017. Accepted 21 November 2017.

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This article is one of a series of invited papers arising from the symposium "Spineless tales: development and evolution of invertebrates" that was co-sponsored by the Canadian Society of Zoologists and the *Canadian Journal of Zoology* and held during the Annual Meeting of the Canadian Society of Zoologists at the University of Western Ontario, London, Ontario, 9–13 May 2016.

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1990; Wilson 2003). Minor workers perform the majority of tasks within a colony, including foraging, brood care, and nest maintenance, whereas soldiers usually perform defense and food processing (Wilson 1985a; Hölldobler and Wilson 1990; Brown and Treniello 1998; Sempo and Detrain 2010). Physically, minor workers are smaller than soldiers; however, the defining feature of the soldier subcaste lies with its head (Hölldobler and Wilson 1990; Wilson 2003; Pie and Treniello 2007). Although soldiers are relatively larger in size, their heads are disproportionately larger (Hölldobler and Wilson 1990; Wilson 2003; Pie and Treniello 2007). These massive heads empower soldiers to perform tasks that require the strength of their large jaws, like the crushing of seeds for food (Hölldobler and Wilson 1990; Huang 2012). The difference is even more dramatic in those *Pheidole* species that have evolved a third supersoldier subcaste with even larger heads (Wilson 2003; Huang 2012). For these reasons, the evolution of soldiers is thought to be a key factor contributing to *Pheidole*'s success and has even led to the group's nickname the big-headed ants (Hölldobler and Wilson 1990; Wilson 2003).

Myrmecologists have primarily focused on the external size differences between *Pheidole* subcastes to understand morphological evolution of the subcastes within this genus (Hölldobler and Wilson 1990; Wilson 2003; Pie and Treniello 2007; Keller et al. 2014; Sarnat et al. 2016; Sarnat et al. 2017). However, recent advances in imaging technology have provided many insights into the differences in internal anatomy of the *Pheidole* subcastes (Seid and Treniello 2005; Seid et al. 2005, 2008; Muscedere et al. 2011a; Ilies et al. 2015). Because of their importance in executing tasks required for division of labour, descriptions of internal anatomical and physiological differences have focused on muscle and brain anatomy (Seid and Treniello 2005; Seid et al. 2005, 2008; Muscedere et al. 2011a; Ilies et al. 2015). However, total volume differences in cephalic glands, as well as muscles and the nervous system, remain understudied.

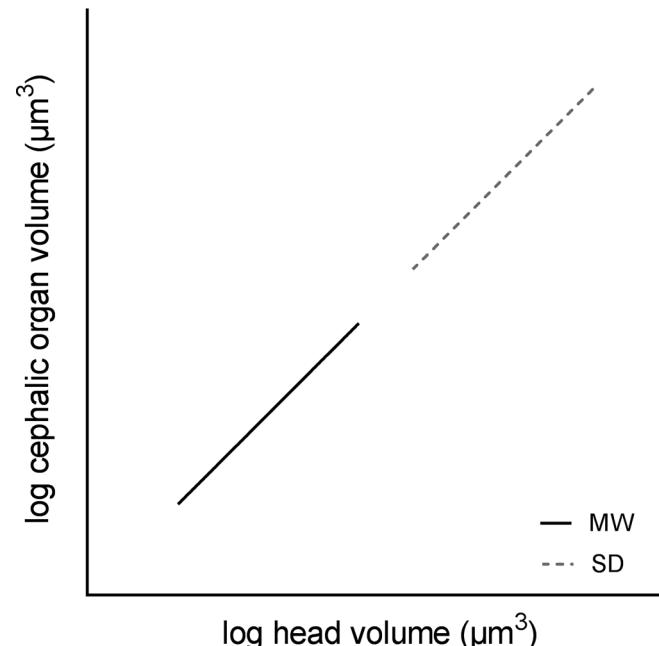
To identify whether disproportionate size differences in subcastes corresponds to disproportionate size differences in internal anatomy, we compared the heads of minor workers and soldiers from four *Pheidole* species (*Pheidole dentata* Mayr, 1886; *Pheidole hyatti* Emery, 1895; *Pheidole navigans* Forel, 1901; *Pheidole spadonia* Wheeler, 1915) that have a broad phylogenetic distribution within *Pheidole*. Specifically, we used microcomputed tomography (microCT) imaging to reconstruct the cephalic glands (mandibular, propharyngeal, and postpharyngeal), pharynx, esophagus, nervous system, and muscle. In doing so, we compared the absolute volume of these internal structures between minor workers and soldiers, as well as these internal structures relative to head size (i.e., intercept) and scaling relationship between the volume of internal structures and the head (i.e., slope). We predicted that the internal organs of soldiers should be bigger than minor workers in absolute volume, but the relative volume and scaling relationship of these cephalic organs to the head should remain the same (i.e., isometric) among subcastes (Fig. 1).

## Materials and methods

### Ant collection and colony care

We collected whole colonies of *P. hyatti* from Pinto Creek, Arizona, USA; *P. dentata* from Gainesville, Florida, USA; and *P. navigans* from Gainesville, Florida, USA. *Pheidole spadonia* are from Tucson, Arizona, USA. The colonies were kept in plastic boxes lined with Fluon®. Artificial nests were made from glass test tubes filled half-way with water and plugged with cotton. Ants were fed mealworms, fruit flies, sugar water, and Bhatkar-Whitcomb diet (Bhatkar and Whitcomb 1970). Colonies were kept in a Conviron environmental chamber (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) maintained at 27 °C, 70% humidity, and 12 h day : 12 h night cycle.

**Fig. 1.** Predicted relationship of cephalic organs to the head in minor workers and soldiers of the ant genus *Pheidole*. Minor workers (MW; black solid line) and soldiers (SD; grey broken line) are predicted to have similar intercepts and an isometric scaling relationship.



### MicroCT preparation and analysis

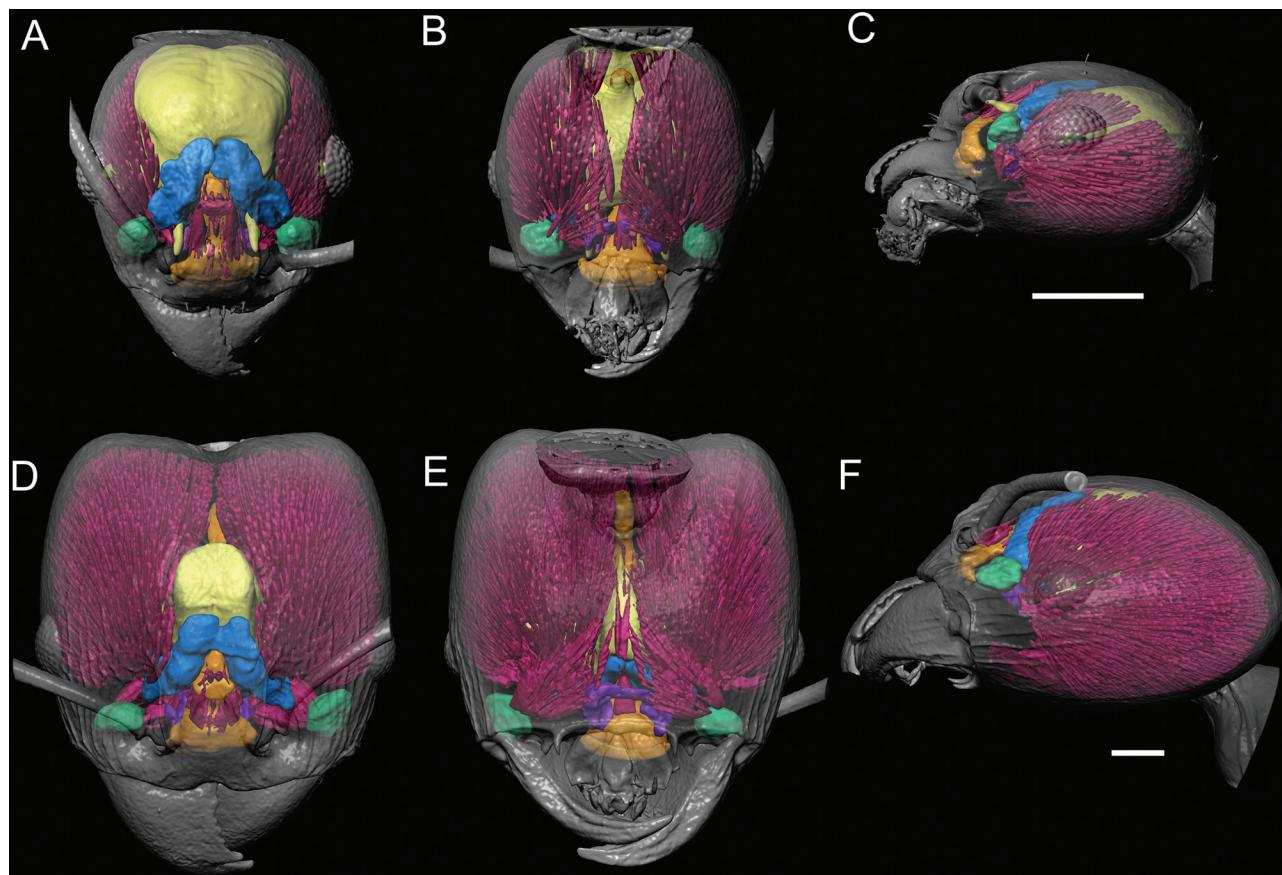
To quantify subcaste differences in size allocation to the different cephalic structures, we examined the heads for our four species with microCT analysis. Our study included two minor workers and two soldiers from each species because microCT analysis is a time-consuming and expensive process. Ants were chosen at random from each colony and fixed in alcoholic Bouin's solution (1:1 Bouin's:ethanol) for 3–6 days. These samples were then transferred to ethanol (96%–100%) for at least 1 day and then stained overnight or longer in 1% iodine in ethanol (I2E) (Metscher 2009). For microCT imaging, we transferred samples to ethanol and mounted in 0.5% agarose in 200 μL micropipette tips (Metscher 2011). High-resolution scans were made with the Zeiss/Xradia MicroXCT X-ray microtomography system in the Department of Theoretical Biology, University of Vienna. This system uses a tungsten source and secondary optical magnification of the scintillation detectors. Projection images were collected at 0.2° steps over a half rotation, with a source voltage of 80 kV at 50 μA (4 W), no beam filter, and exposure times of 8–15 s with 10x magnification or 28–50 s with 20x magnification. Tomographic sections were reconstructed with isotropic voxel sizes of 2.0–4.4 μm using the Xradia XMReconstructor software and volume images were exported as stacks of 8-bit or 16-bit TIFFs.

### Head reconstructions and analysis

We created three-dimensional reconstructions using Imaris x64 version 8.2.0 (Bitplane AG, Zurich, Switzerland). These reconstructed images were arranged with Adobe Illustrator CS5 for figures. We used a paired t test to assess whether there are statistically significant differences between soldiers and minor workers across the four *Pheidole* species in the volume of internal head structures. To determine whether any significant differences were due to caste and (or) head capsule volume, we performed an ANCOVA analysis on the regression of the scaling relationship between each organ and head size and intercept.

Because we are comparing internal anatomy across four broadly distributed species, we needed to account for phylogenetic history, which may introduce phylogenetic nonindependence.

**Fig. 2.** Internal anatomy of minor worker and soldier heads in the ant *Pheidole dentata*. The dorsal minor worker (A), ventral minor worker (B), lateral minor worker (C), dorsal soldier (D), ventral soldier (E), and lateral soldier (F) are shown. Orange represents the esophagus–pharynx, green represents the mandibular glands, red represents muscle, yellow represents the nervous system, blue represents the postpharyngeal glands, and purple represents the propharyngeal glands. Orientation and scale for each figure are depicted with the external image of the head in the top right-hand corner. Scale bars = 100  $\mu\text{m}$  (A–C) and 80  $\mu\text{m}$  (D–F).



dence among species data points. To test whether each species can be treated as a statistically independent data point for the paired *t* test, we used Abouheif's test of phylogenetic independence (Abouheif 1999) for each trait and applied it to the *Pheidole* phylogeny of Moreau (2008), using 1000 permutations to calculate the C-mean. For Abouheif's test, we a priori used a more conservative *p* value of 0.10 to assess phylogenetic nonindependence because of the low number of species (Abouheif 1999).

## Results

### Overview of internal head anatomy in *Pheidole*

We found six structures in the heads of all species: muscle tissue, the nervous system, the esophagus–pharynx, the propharyngeal glands, the postpharyngeal glands, and the mandibular glands (Figs. 2A–2F, 3A–3F, 4A–4F, 5A–5F). The pharynx is located in the anteriormost part of the head at the base of its mouthparts. The posterior of the pharynx is connected to the esophagus, which is a simple tubular structure that runs through to the posteriormost part of the head. These digestive structures are connected; therefore, we will refer to them together as the esophagus–pharynx. The esophagus is paired with a second tubular structure, the ventral nerve cord (VNC), that runs ventral to the esophagus. The VNC is connected anteriorly to the central brain. Optic nerves extend distally from the brain to the ommatidia. Antennal nerves were also found to extend into the antennae from the brain. Because the brain, VNC, optic nerves, and antennal nerves are connected by nervous tissue, we will refer to them together as the nervous system. The propharyngeal glands are small structures

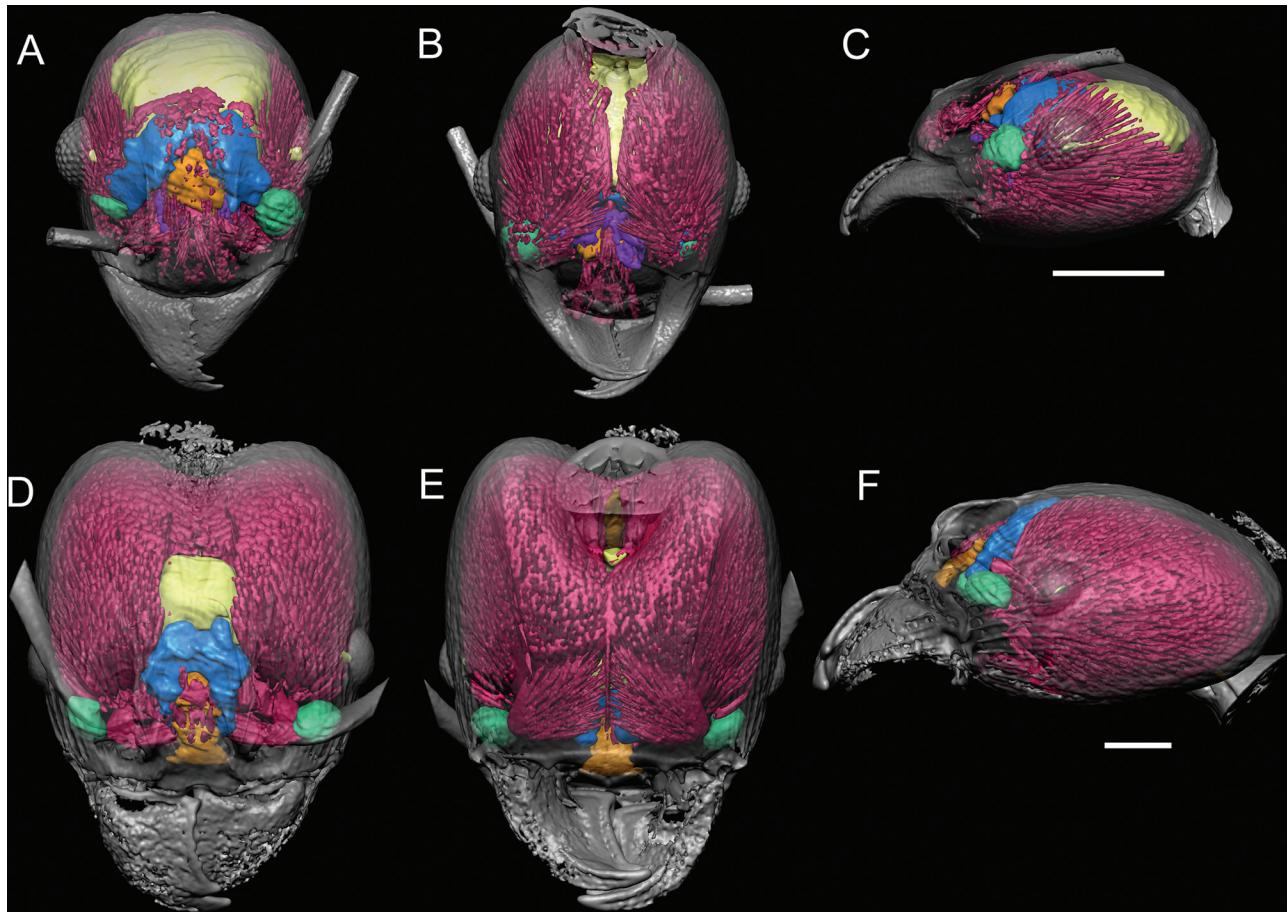
located posterior to the pharynx, whereas the postpharyngeal glands are large sac-like structures located posterior to the pharynx and ventral to the anteriormost esophagus. The mandibular glands are spherical structures on either side of the head, posterior to the clypeus. The muscle extends from just behind the mandibular glands to fill the remainder of the head cavity, dorsally, ventrally, and posteriorly. A second bundle of muscles extends into the head capsule from the ventral mandibular glands and a third bundle of muscles is located dorsal to the pharynx.

### Allocation of head space for each subcaste across species

We first verified whether our data were phylogenetically independent. To do this, we performed Abouheif's test and found that all traits are phylogenetically independent ( $p > 0.10$ ; Table 1). We next asked whether there were differences in the absolute volumes of these organs among subcastes. When we pooled all minor worker and soldier measurements from each species to assess these differences, we found that the muscle was absolutely larger in soldiers, whereas all other cephalic organs (i.e., mandibular glands and propharyngeal glands, propharyngeal glands, postpharyngeal glands, nervous system, and esophagus–pharynx) were absolutely smaller (Figs. 6A, 6C, 6E, 7A, 7C, 7E). Owing to the small sample size, the difference between subcastes was found to be significant for muscle ( $p = 0.0016$ ; Figs. 7A, 7B) and the nervous system ( $p = 0.0028$ ; Figs. 7C, 7D).

Because differences in cephalic organ volume may be attributed to the difference in head size between minor workers and soldiers, we tested if the relative volume (i.e., intercept) and scaling

**Fig. 3.** Internal anatomy of minor worker and soldier heads in the ant *Pheidole hyatti*. The dorsal minor worker (A), ventral minor worker (B), lateral minor worker (C), dorsal soldier (D), ventral soldier (E), and lateral soldier (F) are shown. Orange represents the esophagus–pharynx, green represents the mandibular glands, red represents muscle, yellow represents the nervous system, blue represents the postpharyngeal glands, and purple represents the propharyngeal glands. Orientation and scale for each figure are depicted with the external image of the head in the top right-hand corner. Scale bars = 100  $\mu\text{m}$  (A–C) and 100  $\mu\text{m}$  (D–F).



relationship (i.e., slope) of these structures to the head was the same among subcastes using an ANCOVA analysis. We found that muscle was also relatively larger in soldiers, whereas all other cephalic organs were relatively smaller (Figs. 6B, 6D, 6F, 7B, 7D, 7F). Owing to the small sample size, we found a significant difference between subcastes only for the propharyngeal glands ( $p = 0.034$ ), the nervous system ( $p = 0.014$ ), and the esophagus–pharynx ( $p < 0.0001$ ). Finally, we found that the scaling relationship of the mandibular glands, postpharyngeal glands, muscle, and esophagus–pharynx to the head were very similar among subcastes (Figs. 6B, 6D, 6F, 7B, 7F). The nervous system was the only organ found to have an allometric relationship with head size among subcastes (Fig. 7D). We did not find a statistically significant relationship for our slope comparisons.

## Discussion

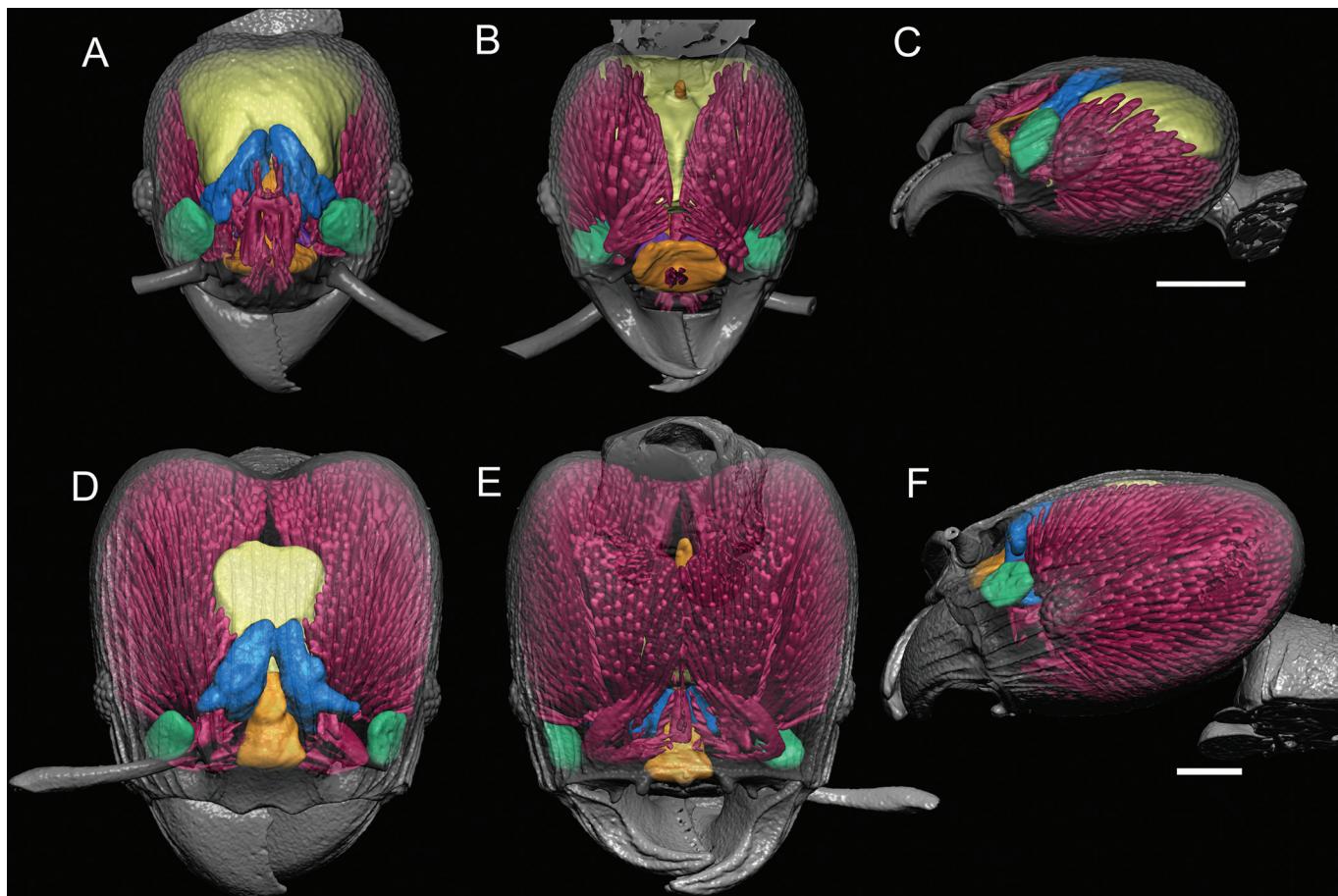
The distinction between a *Pheidole* minor worker and soldier lies in the soldier's disproportionately larger head. The goal of our study was to elucidate the extent of this relationship by determining whether the absolute size, relative size, and scaling of internal cephalic structures to the head differed among subcastes as well. Our null hypothesis was that soldiers would have larger internal structures, which would scale with the head accordingly. Surprisingly, we found that most cephalic structures, with the exception of muscle, were absolutely and relatively smaller in soldiers than in minor workers. We note that although many of the subcaste

comparisons are not significantly different, our limited sample size puts us at risk of a type II error, whereby we erroneously do not reject the null hypothesis. Because of this risk, we discuss the size and scaling relationships found and suggest hypotheses for future studies to test with greater sample sizes.

### Nervous system

*Pheidole* brains are compartmentalized into a variety of structures that integrate sensory information, direct motor functions, and process higher level operations like learning and memory (Muscadere and Traniello 2012; Ilieş et al. 2015). Of all the internal cephalic anatomy that we analyzed, the brain has received the most attention in recent years because of its importance to behavioural flexibility and division of labour. For example, as minor workers mature and expand their behavioural repertoire, they undergo remodelling of synapses (Seid et al. 2005), altering of biogenic amines (Seid and Traniello 2005), and increasing immunoreactivity to serotonin (Seid et al. 2008). These changes primarily affect neuronal networks associated with learning and memory (mushroom bodies), sensing (antennal lobes), and the central nervous system (Seid et al. 2008), which are important for behavioural task performance. Similar associations have been made for *Pheidole* subcastes. Muscadere and Traniello (2012) compared dissected brains of minor workers and soldiers from *P. dentata*, *Pheidole morrisi* Forel, 1886, and *Pheidole pilifera* (Roger, 1863). These species broadly differ with respect to life history, colony size,

**Fig. 4.** Internal anatomy of minor worker and soldier heads in the ant *Pheidole navigans*. The dorsal minor worker (A), ventral minor worker (B), lateral minor worker (C), dorsal soldier (D), ventral soldier (E), and lateral soldier (F) are shown. Orange represents the esophagus–pharynx, green represents the mandibular glands, red represents muscle, yellow represents the nervous system, blue represents the postpharyngeal glands, and purple represents the propharyngeal glands. Orientation and scale for each figure are depicted with the external image of the head in the top right-hand corner. Scale bars = 100 µm (A–C) and 100 µm (D–F).



subcaste composition, and behavioural plasticity (Muscедере and Тranieлlo 2012). When controlling for brain size, Muscedere and Тranieлlo (2012) found that the brain was configured as a mosaic and different subregions of the brain varied in scale between minor workers and soldiers (subesophageal ganglion, antennal lobe, and mushroom bodies). Furthermore, they found that the central brain volume relative to head width was larger for minor workers than for soldiers and varied among species (Muscедере and Тranieлlo 2012). Although the brain is a mosaic, soldiers tend to have more integrated brains than minor workers, with a strong effect of species ecotype and sociobiology (Muscедере and Тranieлlo 2012; Ilieš et al. 2015).

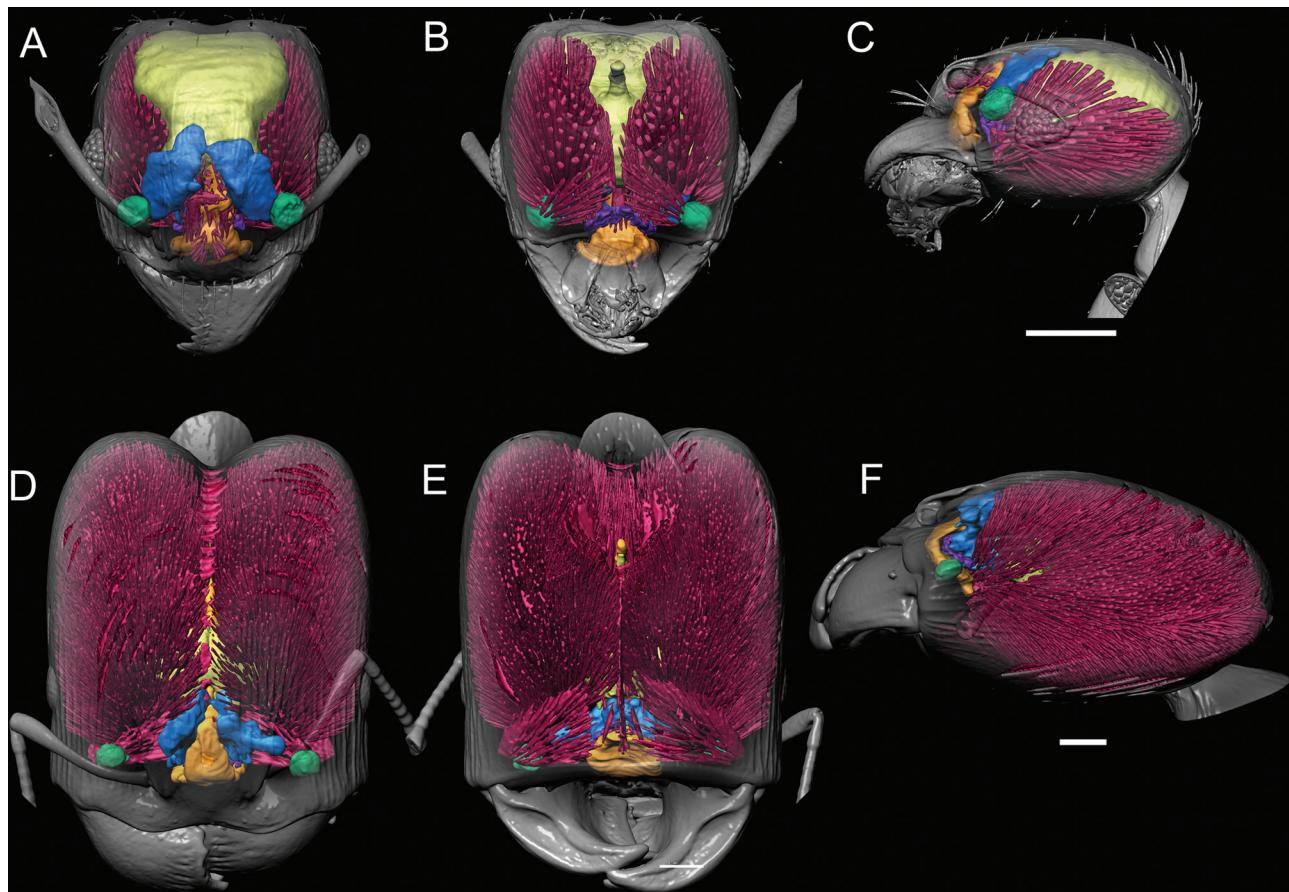
Our study finds a similar pattern using the absolute and relative nervous system volume, wherein minor workers allocate more space to the nervous system than soldiers. Furthermore, we find that the scaling relationship of the nervous system to the head is not isometric, but instead is allometric, indicating that there may be a functional explanation for this difference in allocation. For example, minor workers may require more brain capacity to perform complex tasks in the nest. Of course, our data are a broad look at all the nervous tissue in the head and we did not make a distinction between subregions of the brain or separate nerves from the central brain so we cannot comment on the degree of brain modularity for the species that we studied. We believe that this result will require further exploration including similar comparisons to Muscedere and Тranieлlo (2012) and Ilieš et al. (2015) with respect to morphology, behavioural plasticity, task division,

and the selective pressures (i.e., ecotype and sociobiology) in evolutionary history that may have led to this striking difference.

#### Muscle

According to Gronenberg et al. (1997), the mandibles of insects are akin to the hands of humans because they are both indispensable appendages adapted for a variety of roles. In ants, not only can the mandibles themselves evolve different shapes to specialize on particular tasks but also the head muscle can evolve to control mandibular velocity and force for task specialization (Gronenberg et al. 1997). For example, predators like the Indian jumping ant (*Harpegnathos saltator* (Jerdon, 1851)) have fast-acting muscles that allow the mandibles to quickly catch prey (Gronenberg et al. 1997). In *Pheidole*, soldiers are thought to have large heads and complementary large mandibles to defend the colony against competitors and to mill seeds. For these roles, *Pheidole* do not require rapid mandibular action, but instead rely on cephalic mandibular closer muscles to provide the force to carry out powerful crushing actions (Gronenberg et al. 1997; Huang 2012). Muscedere et al. (2011b) compared three different cephalic muscle groups in *P. dentata*: the mandibular closer muscles, which are important for nursing, foraging, and defense; the pharynx dilator muscles, which are important for feeding; the antennal muscles, which are important for collecting sensory stimuli. They found that *P. dentata* soldiers have thicker muscle fibres than minor workers and that older workers generally have thicker muscle fibres than younger workers. We found that soldiers in the four

**Fig. 5.** Internal anatomy of minor worker and soldier heads in the ant *Pheidole spadonia*. The dorsal minor worker (A), ventral minor worker (B), lateral minor worker (C), dorsal soldier (D), ventral soldier (E), and lateral soldier (F) are shown. Orange represents the esophagus–pharynx, green represents the mandibular glands, red represents muscle, yellow represents the nervous system, blue represents the postpharyngeal glands, and purple represents the propharyngeal glands. Orientation and scale for each figure are depicted with the external image of the head in the top right-hand corner. Scale bars = 100 µm (A–C) and 100 µm (D–F).



**Table 1.** No phylogenetic signal in head traits of the ant genus *Pheidole*.

Subcaste	Structure	C-mean	p
Minor worker	Esophagus–pharynx	-0.21	0.17
	Mandibular glands	0.0033	0.42
	Muscle	0.2	0.32
	Nervous system	0.24	0.24
	Postpharyngeal glands	-0.17	0.32
	Propharyngeal glands	-0.086	0.48
Soldier	Esophagus–pharynx	0.11	0.31
	Mandibular glands	0.046	0.38
	Muscle	-0.25	0.12
	Nervous system	0.076	0.56
	Postpharyngeal glands	-0.12	0.24
	Propharyngeal glands	0.12	0.47

Note: C-mean and p values generated using Abouheif's test of phylogenetic independence.

species studied, including *P. dentata*, have more muscle by absolute volume than minor workers, supporting the findings of Muscedere et al. (2011b). Our findings suggest that muscle scales proportionately with the head, although their relative size to the head may be greater in soldiers. This result strengthens the notion that muscle has played a vital role in the diversification of caste specialization in *Pheidole*. Because *Pheidole* soldiers perform a variety of tasks related to mandible use like seed crushing and combat, it will be important to elucidate how subtle differences in

muscle (e.g., fibre thickness and muscle type) may relate to different tasks by comparing species with diverse ecological lifestyles and varied measures of musculature development.

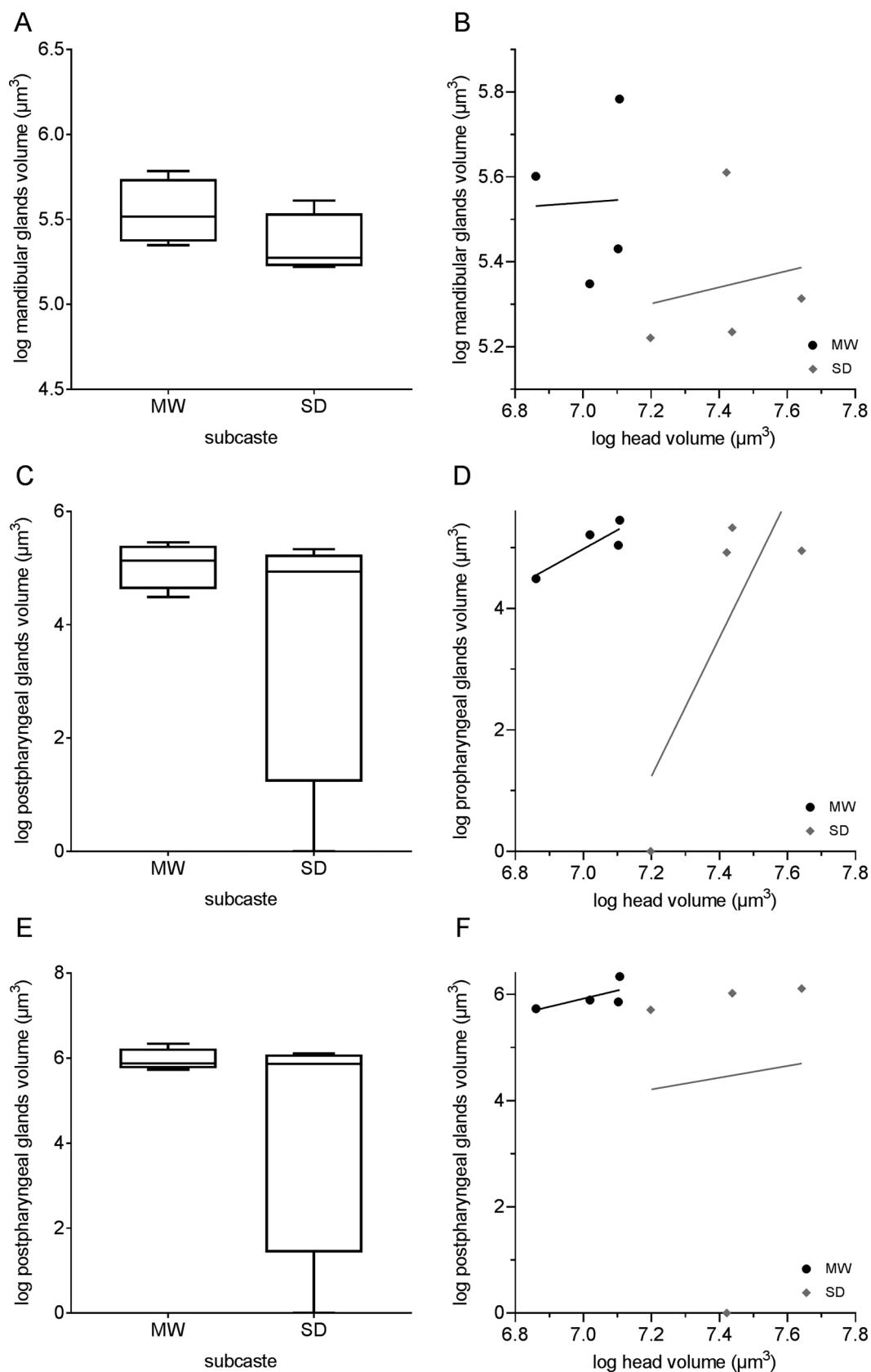
#### Digestive organs

Food consumption is a vital process for all organisms, yet the structures that enable this process have been largely overlooked in *Pheidole*. We analyzed three digestive structures in the head: the propharyngeal glands, the postpharyngeal glands, and the esophagus–pharynx, which are together an integral part of food processing and produce enzymatic molecules that aid digestion (Fluri et al. 1982; Jackson and Morgan 1993; Eelen et al. 2006; Billen 2009). We found that all three digestive organs are absolutely and relatively smaller in soldiers, but appear to be isometric between subcastes. If soldiers allocate less cephalic space to food-processing structures, then this may reflect their limited capacity for food processing in the colony, beyond severing large insects and grains.

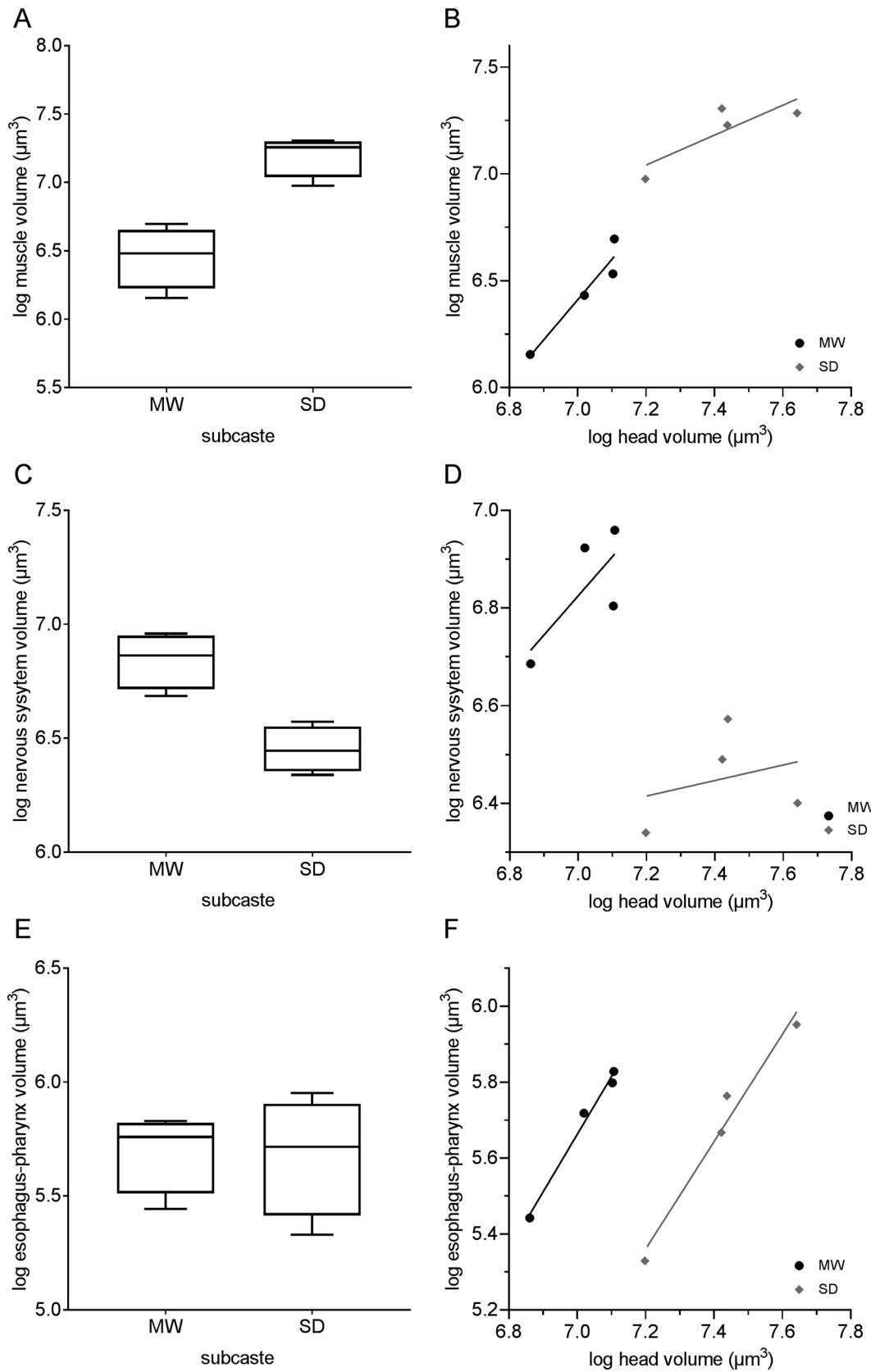
#### Mandibular glands

Cephalic glands are important for production of digestive enzymes and social pheromones, including alarm and recognition pheromones that influence worker behaviour and physiology (Attygalle and Morgan 1984; Ali et al. 1988; Billen 2009). Cephalic mandibular glands are among the most diverse glands found in all insects (Attygalle and Morgan 1984; Ali et al. 1988; Billen 2009). In ants, mandibular glands have taken on both defensive and pheromonal functions, producing a variety of chemicals including sulphides, alcohols, ketones, aldehydes, terpenes, and benzenoids

**Fig. 6.** Size of cephalic glands in minor workers and soldiers of the ant genus *Pheidole*. Volume for the mandibular glands (A), propharyngeal glands (C), and postpharyngeal glands (E) is expressed in a logarithmic scale by subcaste. Relative size and scaling of the mandibular glands (B), propharyngeal glands (D), and postpharyngeal glands (F) to the head is shown for each subcaste. Minor workers (MW) are represented by black circles and soldiers (SD) are represented by grey diamonds.



**Fig. 7.** Size of other cephalic organs in minor workers and soldiers of the ant genus *Pheidole*. Volume for the muscle (A), nervous system (C), and esophagus-pharynx (E) is expressed in a logarithmic scale by subcaste. Relative size and scaling of the muscle (B), nervous system (D), and esophagus-pharynx (F) to the head is shown for each subcaste. Minor workers (MW) are represented by black circles and soldiers (SD) are represented by grey diamonds.



(Attygalle and Morgan 1984). In myrmecines specifically, these glands have largely taken a defensive role, producing alarm pheromones that prime defensive behaviour (Attygalle and Morgan 1984). Mandibular glands may also play a potential role in producing developmental pheromones. Soldiers play a major role in the regulation of subcaste development by producing a soldier-inhibitory pheromone (Gregg 1942; Passera 1974; Wheeler and Nijhout 1984; Lillico-Ouachour and Abouheif 2017). Currently, we do not know where the soldier-inhibitory pheromone is produced, but the mandibular glands have long been a candidate because of their versatility and connection to similar pheromones in other insects (Attygalle and Morgan 1984; Lefeuve and Bordereau 1984; Tarver et al. 2009, 2011; Matsuura et al. 2010). If the mandibular glands produce a defensive or socio-developmental pheromone specific to soldiers, then soldiers would likely have larger mandibular glands by volume and the mandibular glands would likely have a steeper allometric relationship with head size compared with that of minor workers. Surprisingly, we found that that mandibular glands were absolutely and relatively smaller by volume in the soldiers than in minor workers, whereas their scaling relationship was the same in both subcastes. Because the mandibular glands are smaller in soldiers, this would suggest that the mandibular glands are less likely to be the site of the production of the soldier-inhibitory pheromone or have a specialized defensive role. Research that details the compounds produced by the mandibular glands in soldiers and minor workers, as well as their pheromonal function, will need to be conducted to test this hypothesis.

## Conclusion

Our study marks the first step in describing the internal head anatomy of subcaste heads in *Pheidole*. We found that the relationship between these internal structures is not simply an isometric scaling up from minor workers to soldiers. Overall, the soldiers appear to have more muscle than minor workers, but less cephalic glands, nervous system, and digestive organs in terms of relative and absolute volume. One possibility is that this is an individual-level trade-off owing to energy limitation, where some organs grow at the expense of others. In our study, this would mean that soldiers have more muscle at the expense of the nervous system. Alternatively, our data may suggest that this is a morphological specialization and division of labour of internal anatomy at the colony level. As *Pheidole* are highly eusocial, it is more likely that our findings reflect a functional specialization of internal anatomy among castes. For example, we found that minor workers have more cephalic nervous system than soldiers, with a steeper allometric relationship to the head. This may reflect the specialization of minor workers on complex tasks like foraging and brood rearing in the colony. We also found that soldiers have more muscle than minor workers. This may reflect the ability of soldiers to specialize on defense, cracking seeds, and dismantling of food. When minor workers are depleted in a colony, soldiers can take on their role. We propose that soldiers retain a reduced nervous system and other cephalic structures like the mandibular glands to maintain this behavioural flexibility. Future studies of internal anatomy across a greater number of *Pheidole* species, combined with functional and behavioural studies, will be able to test these alternative hypotheses.

## Acknowledgements

We thank the Abouheif Laboratory for comments on the manuscript, as well as L. Davis, Ray Sanwald, M. Huang, D. Wheeler, and R. Johnson for their invaluable help with ant collection. This work was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant and the McGill University Tomlinson Science Award to E.A., as well as an NSERC Canada Graduate Scholarship (Master's) to A.L.O.

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