



# Evidence for a thoracic crop in the workers of some Neotropical *Pheidole* species (Formicidae: Myrmicinae)

A. Casadei-Ferreira<sup>a,\*</sup>, G. Fischer<sup>b</sup>, E.P. Economo<sup>b</sup>

<sup>a</sup> Departamento de Zoologia, Universidade Federal do Paraná, Avenida Francisco Heráclito dos Santos, s/n, Centro Politécnico, Curitiba, Mailbox 19020, CEP 81531-980, Brazil

<sup>b</sup> Biodiversity and Biocomplexity Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna, Okinawa, 904-0495, Japan

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## ABSTRACT

The ability of ant colonies to transport, store, and distribute food resources through trophallaxis is a key advantage of social life. Nonetheless, how the structure of the digestive system has adapted across the ant phylogeny to facilitate these abilities is still not well understood. The crop and proventriculus, structures in the ant foregut (stomodeum), have received most attention for their roles in trophallaxis. However, potential roles of the esophagus have not been as well studied. Here, we report for the first time the presence of an auxiliary thoracic crop in *Pheidole aberrans* and *Pheidole deima* using X-ray micro-computed tomography and 3D segmentation. Additionally, we describe morphological modifications involving the endo- and exoskeleton that are associated with the presence of the thoracic crop. Our results indicate that the presence of a thoracic crop in major workers suggests their potential role as repletes or live food reservoirs, expanding the possibilities of tasks assumed by these individuals in the colony. Our contribution emphasizes the utility of combining data from external and internal morphology to better understand functional and behavioral mechanisms.

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## 1. Introduction

One of the most noteworthy behaviors in social insects (e.g. ants, bees, termites, wasps) is food sharing among nestmates (adult/brood and adult/adult) (Hölldobler and Wilson, 2009). Solid food, such as seeds and prey, are shared among the brood of the colony and typically consumed freely (Hölldobler and Wilson, 1990, 2009). Liquid food, however, is carried to the nest by forager workers in the crop ("social stomach") and later regurgitated to nestmates, and thus distributed over the colony (Hölldobler and Wilson, 1990). This process of liquid food storage and its subsequent sharing among members of a colony is known as stomodeal trophallaxis (Wheeler, 1910; Hölldobler and Wilson, 1990; Greenwald et al., 2015; LeBoeuf et al., 2016; Schultner et al., 2017). In these insects, the crop is a pear-shaped bag with thin and chitinous walls, without any associated gland and incapable of absorbing food, serving as a storage for the fluid obtained by these individuals (Wheeler, 1910; Chapman and Chapman, 2013; Snodgrass, 1935). Contrary to other groups of insects (e.g. Orthoptera, Blattodea, Coleoptera, etc.) where the crop normally resides entirely within the

thoracic cavity, ants and other hymenopterans have a constriction between the first and second abdominal segments and the crop resides exclusively inside the abdomen (abdominal segments III–VII when the waist consists of a single isolated segment; abdominal segments IV–VII when the waist consists of two isolated segments) (Wheeler, 1910; Snodgrass, 1935, 1956).

Considering ants (Hymenoptera: Formicidae), in which workers and queens feed exclusively on liquids, stomodeal trophallaxis is the most common strategy of food exchange in the colony (Hölldobler and Wilson, 1990, 2009). The crop of most ant species is capable of considerable distension, in extreme cases, certain workers act as live reservoirs during lean periods (e.g. *Myrmecocystus* Wesmael) which are commonly referred to as "repletes" (Glancey et al., 1973; Rissing, 1984; Hölldobler and Wilson, 1990; Børgesen, 2000). The crop, as part of the foregut (stomodeum), has mainly a mechanical function but also acts as a physiological adjunct (Snodgrass, 1935; Chapman and Chapman, 2013). Its physiological function relates to its capacity to increase the space available for digestive purposes (Snodgrass, 1935). Thus, digestion occurs partially by the action of salivary liquid mixed with the food during ingestion as well as with gastric juices that flow forward from the ventriculus (Snodgrass, 1935).

In ants, the mouthparts, the anterior portion of the foregut – mostly the pharynx and preoral cibarium – and the posterior portion including the crop and proventriculus, have been extensively

\* Corresponding author.

E-mail addresses: [alexandreferreira@gmail.com](mailto:alexandreferreira@gmail.com) (A. Casadei-Ferreira), [georgf81@gmail.com](mailto:georgf81@gmail.com) (G. Fischer), [evaneconomo@gmail.com](mailto:evaneconomo@gmail.com) (E.P. Economo).

explored (Lanan et al., 2016; Vieira et al., 2017; Fujioka and Okada, 2019; Paul and Roces, 2019; Probst et al., 2019; Richter et al., 2019). For instance the proventriculus, the structure controlling liquid flow between the crop and the midgut, varies in shape distinctively enough to provide useful characters in a phylogenetic context at generic and tribal levels (Eisner and Brown, 1958).

In contrast, the esophagus remains largely unexplored, being described merely as a narrow part of the stomodeum between the pharynx and crop, serving exclusively as an area for food passage (Snodgrass, 1935; Chapman and Chapman, 2013). The esophagus has usually a simple tubular form in ants. In workers, this structure narrows as it travels through the mesosoma towards the gaster, while in males and queens its diameter remains mostly unchanged, through the developed wing muscles and glands in the mesosoma to the gaster, where the most important parts are situated (e.g. crop, proventriculus and hindgut [proctodeum]) (Wheeler, 1910). Indeed, most of the literature involving the anatomy of the digestive tract of ants, which considers the esophagus, was published during the 1980s and 1990s. As an example, the dilatation of the esophagus, known as thoracic crop (i.e. structure with potential analogous function to the crop, but not homologous), was reported by Petersen-Braun and Buschinger (1975) in physogastric queens of Myrmicinae (*Monomorium pharaonis* [Linnaeus], *Myrmica ruginodis* Nylander, *Leptothorax unifasciatus* [Latreille], *Leptothorax interruptus* [Schenck], *Leptothorax affinis* Mayr, and *Stenamma westwoodii* Westwood), and Formicinae (*Lasius niger* [Linnaeus]). A few later studies described the dilatation of the esophagus of workers in several additional subfamilies, including Dolichoderinae (*Dolichoderus attelaboides* [Fabricius]; Caetano et al., 1990), Ectatomminae (*Ectatomma quadridens* [Fabricius]; Caetano et al., 1988), Paraponerinae (*Paraponera clavata* [Fabricius]; Caetano et al., 1986) and Ponerinae (*Dinoponera gigantea* [Perty], *Neoponera villosa* [Fabricius], *Neoponera obscuricornis* [Emery], *Odontomachus bauri* [Emery], *Odontomachus minuta* Luederwaldt, *Odontomachus affinis* Guérin-Méneville, and *Pachycondyla* sp.; Caetano and Lage Filho, 1982; Caetano, 1988; Caetano et al., 1986, 1997). Caetano, 1993 also described the presence of a thoracic crop in males of the ponerine *N. villosa* and the myrmicine *Cephalotes pusillus* (Santschi).

In physogastric queens of *M. pharaonis*, the dilation of the esophagus is associated with ovarian overdevelopment, due to which only little space is available to the distention of the gastral crop, thus allowing the storage of liquids in the mesosoma (Petersen-Braun and Buschinger, 1975). In queens of *Solenopsis invicta* Buren, the thoracic crop occupies the entire (dorsal) thoracic cavity and is filled with a clear yellowish fluid (mainly composed of triglycerides) from wing muscle histolysis, probably due to a redistribution of the contents of the gastral crop (Glancey et al., 1973; Phillips and Vinson, 1980). A pattern like that found in *S. invicta* and in *M. pharaonis* had already been reported previously by Janet (1907) for queens of *L. niger*. In workers of Ponerinae, Ectatomminae, and Paraponerinae, the auxiliary crop is a response to the process of fusion of the abdominal tergite and sternite IV (related to increasing support for the sting muscles), which results in a smaller distension capacity for the gastral crop (Caetano, 1988; Caetano et al., 1990). Because of this reduced space, an expansion of the esophagus is expected to accommodate the liquid reserves. Contrary to this pattern in the subfamily, workers of *Odontomachus* did not have the fusion in the IV abdominal segments (Caetano et al., 1982). Moreover, the necessity to store liquids in most Ponerinae is not comparable with other subfamilies that frequently consume and save liquids resources (e.g. Dolichoderinae) (Hanisch et al., 2020).

Studies involving internal morphological changes associated with food reserves in workers have been largely conducted for

monomorphic species (e.g. some *Myrmecocystus* and *Brachymyrmex* species) (McCook, 1845). However, few cases are reported about the presence of workers acting as live reservoirs in dimorphic species, some examples are *Melophorus bagoti* Lubbock, *Melophorus cowlei* (Froggatt) (Heterick et al., 2017) and *Colobopsis nipponica* (Wheeler) (Hasegawa, 1993), as well as its possible presence in *Carebara* (Fischer et al., 2015). Among the Myrmicinae genera, *Pheidole* is well known by the conspicuous dimorphism between its worker castes, in which major workers differ from minors by their proportionally larger heads and robust body (Wilson, 2003; Traniello, 2010; Casadei-Ferreira, 2019). This dimorphism reflects different functions performed by each sub-caste (Wilson, 2003; Traniello, 2010; Casadei-Ferreira, 2019). Minor workers performing the basic functions such as brood care, nest maintenance, and foraging, while majors are mostly responsible for the defense of the nest and/or resources, as well as for food processing and transport (Wilson, 2003; Traniello, 2010; Casadei-Ferreira, 2019). Additionally, few publications have mentioned the existence of majors functioning as replete workers in *Pheidole* (Wilson, 1984; Tsuji, 1990; Lachaud et al., 1992). Major workers of *Pheidole*, both in the New (e.g. *Pheidole hortensis* Forel, *Pheidole mendicula* Wheeler, and *Pheidole pallidula* [Nylander]) and Old World (i.e. *Pheidole pili* Santschi, *Pheidole nodus* Smith, and *Pheidole ryukyensis* Ogata), were found exhibiting an extremely distended gaster, filled with liquid, as in other repletes (Wilson, 1984; Tsuji, 1990; Lachaud et al., 1992). But little is known about possible alternative routes that workers of *Pheidole* may adopt for storing liquid reserves in their colonies, such as the thoracic crop known in physogastric queens of other ants. Here, using data from X-ray microtomography (microCT), we describe for the first time the existence of a distended esophagus forming an auxiliary crop in workers of two *Pheidole* species, as well as modifications on the internal and external mesosomal anatomy.

## 2. Material and methods

We have explored the internal and external anatomy of several species belonging to the myrmicine genus *Pheidole* with microCT. We describe modifications associated with the thoracic crop that are unique to only two of these species. Specimens of *Pheidole deima* Wilson were collected in French Guiana, Nouragues Field station, Inselberg Camp in 2018. The second species, *Pheidole aberrans* Mayr, was collected at the campus of the Federal University of Paraná, in Curitiba, Brazil.

We used micro-CT/ $\mu$ CT scans created with a ZEISS Xradia 510 Versa and ZEISS Scout and Scan Control System software. Scan settings were selected to yield optimum scan quality: 4 $\times$  objective, exposure times between 1 and 5 s, source-filter “Air”, voltage between 30 and 50 keV, power between 4 and 5 W, and field mode “normal”. The combination of voltage, power, and exposure time was set to yield intensity levels between 15,000 and 17,000 across the whole specimen. Scan times varied from 27 to 50 min, depending on exposure times. Full 360-degree rotations were done with 801 projections. The resulting scans have resolutions of 1013  $\times$  992  $\times$  999 (H  $\times$  W  $\times$  D) pixels, while voxel sizes range between 2.25  $\mu$ m and 5.39  $\mu$ m. 3D reconstruction of the resulting scans was done with XMReconstructor.

The 3D surface models, as well as the snapshots from the scanned slices, were generated with 3D Slicer v. 4.11.0 (Kikinis et al., 2014). Color and light effect were created, and snapshots made in Blender v. 2.82.7 (2020). The high-resolution 2D images used in the plates were obtained from AntWeb (AntWeb.org). All specimens examined in this study have been added to a database of ant images and they are available on Antweb (<http://www.antweb.org>). Each specimen can be traced by a unique specimen identifier attached to

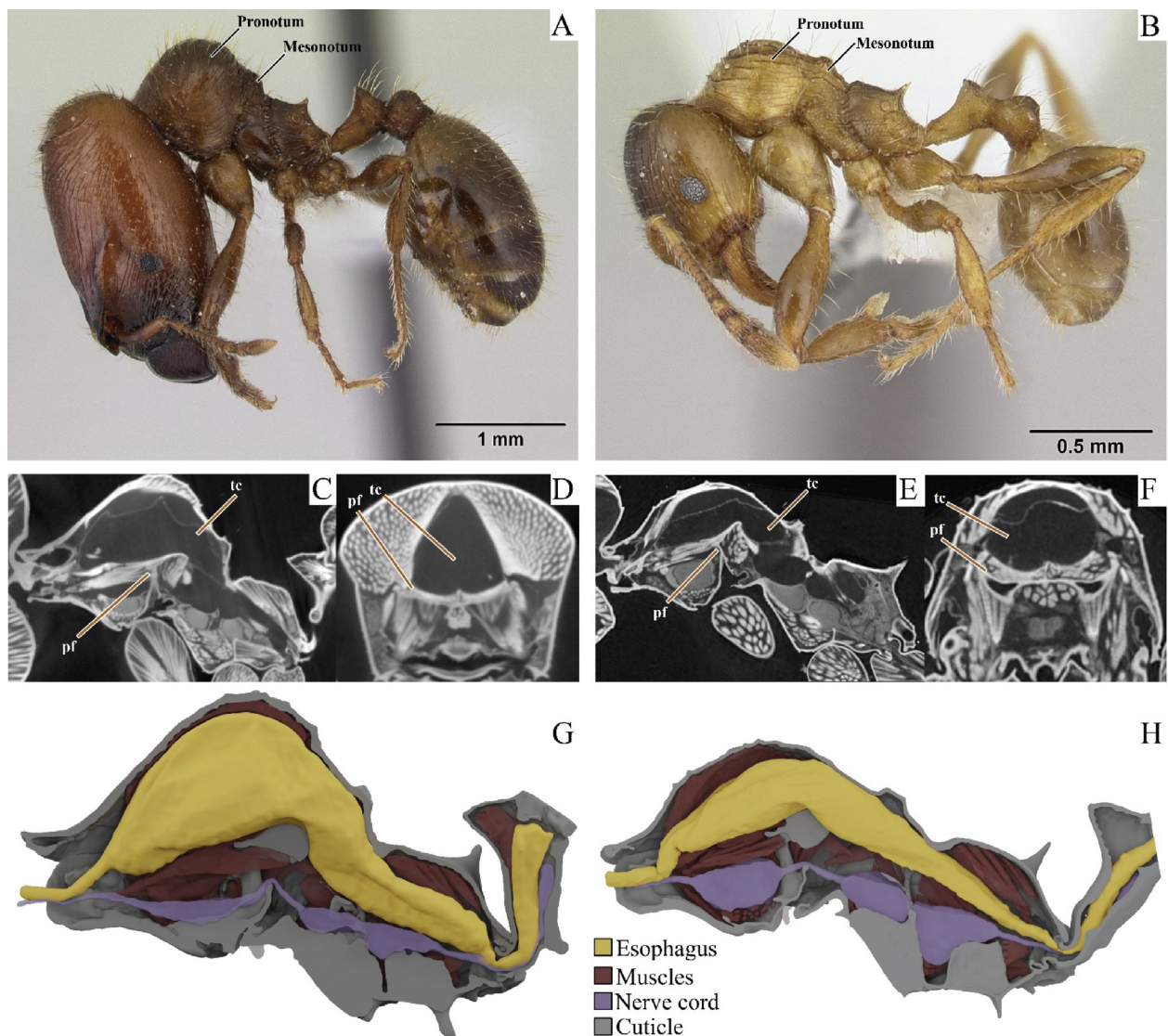
its pin (e.g. CASENT0764125). The datasets provided in this study consist of the full micro-CT original volumetric datasets. All data are freely available in the supplementary material on Dryad (<https://doi.org/10.5061/dryad.p2ngf1vnn>).

### 3. Results and discussion

Majors of *P. aberrans* have the pro- and mesonotum widely fused, convex and prominent (Fig. 1A and C). Additionally, the profurca is broadly curved in these workers (Fig. 1C), creating a large cavity inside the prothorax that is occupied by the dilated esophagus (Fig. 1C and G). In these majors, the pronotum is slightly expanded laterally, which produces a region that houses the muscles associated with the head (Fig. 1D). Majors of *P. deima* have a distinct mesosomal shape, with the mesonotum protruding beyond the height of the pronotum (Fig. 2A), an unusual feature for the genus. This notal projection in *P. deima* creates a large compartment where the esophagus expands (Fig. 2C and G) in addition to the prothorax cavity. Like majors of *P. aberrans*, a lateral expansion in

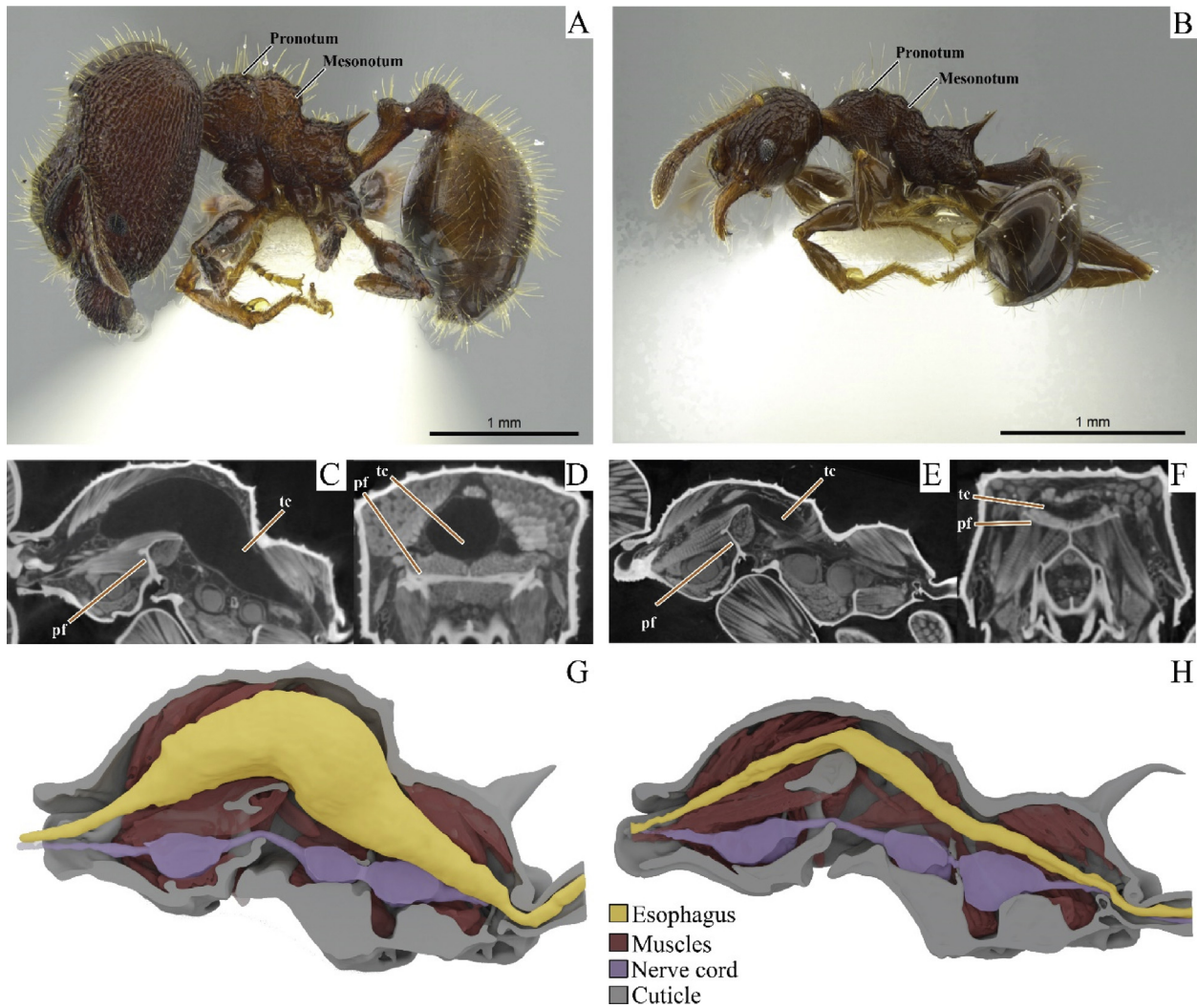
the pronotum also occurs in *P. deima* (Fig. 2D) as well as the curvature in the profurca (Fig. 2C). There are clear differences between the dilated esophagi observed in *P. aberrans* and *P. deima* (Fig. 3A–D) when compared to species in which the esophagus is not dilated, such as in *Pheidole cursor* Wilson (Fig. 3E and F). Additionally, the abdominal crop of major and minor workers of *P. aberrans* (Fig. 3A and B) and *P. deima* (Fig. 3C and D) does not present significant differences from the abdominal crop in workers of *P. cursor* (Fig. 3E and F).

Unlike the major workers, minors of both species do not present those external modifications on the mesosoma (Figs. 1B and 2B). In these individuals, the profurca is relatively less curved (Figs. 1E and 2E), however, this curvature also promotes an increase in the prothoracic cavity (Figs. 1E, F, 2E, F and 3B, D). Minors of *P. aberrans* and *P. deima* have a considerably dilated esophagus (Figs. 1H, 2H and 3B, D) when compared to other species in the genus (Fig. 3F). This dilatation may be associated with a greater capacity for loading liquid resources, which would prevent the foraging capacity from being compromised by an exacerbated dilation of the gaster.



**Fig. 1.** *Pheidole aberrans* thoracic crop. High-resolution images showing the external morphology of (A) major (AntWeb, CASENT0178005; Photographer: April Nobile) and (B) minor worker (AntWeb CASENT0178005; Photographer: April Nobile). Micro-CT 2D slices for major (C [sagittal section] and D [cross section]) and minor worker (E and F) showing the shape of the profurca (pf) and the space occupied by the thoracic crop (tc). Sagittal sections of the segmented 3D models generated from the micro-CT data illustrate the shape of the esophagus in the major (G) and minor worker (H).





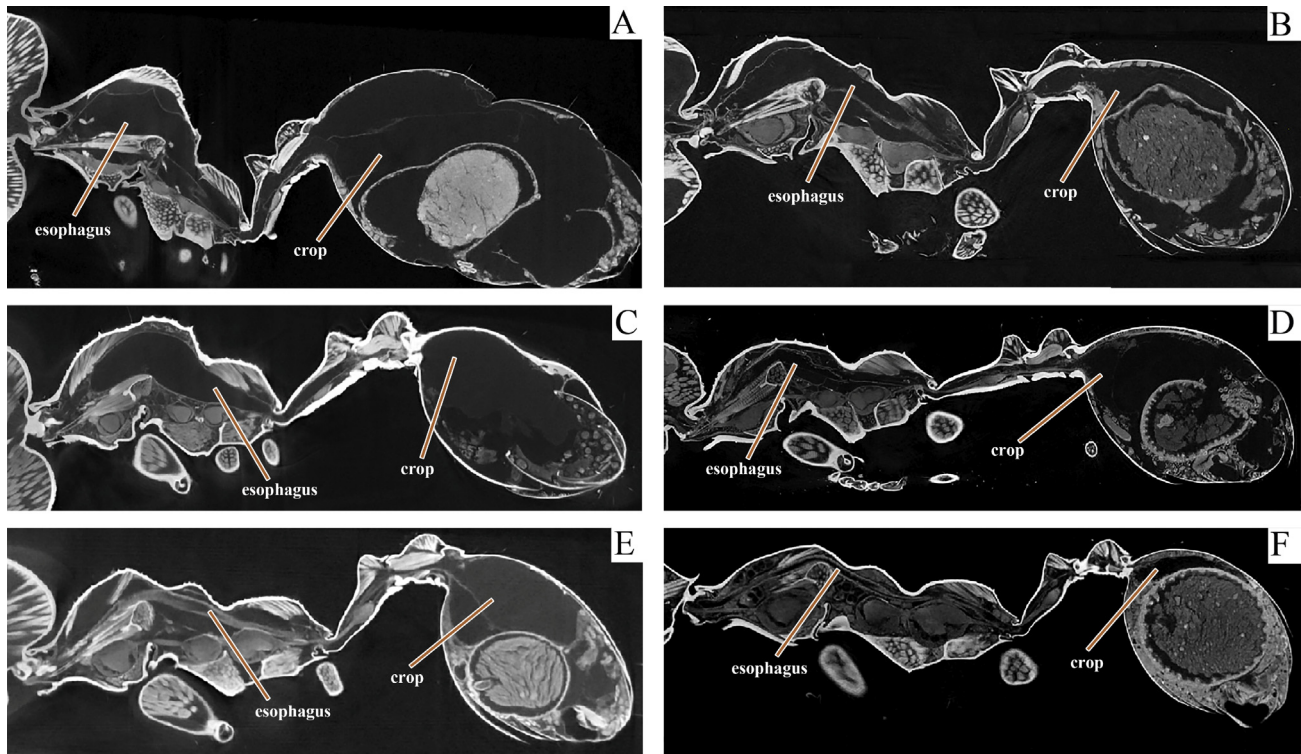
**Fig. 2.** *Pheidole deima* thoracic crop. High-resolution images showing the external morphology of (A) major (Paratype; AntWeb, JTL000016361; Photographer: Jeremy Pillow) and (B) minor worker (Paratype; AntWeb CASENT0627924; Photographer: Jeremy Pillow). Micro-CT 2D slices for major (C [sagittal section] and D [cross section]) and minor worker (E and F) showing the shape of the profurca (pf) and the space occupied by the thoracic crop (tc). Sagittal sections of the segmented 3D models generated from the micro-CT data illustrate the shape of the esophagus in the major (G) and minor worker (H).

The external morphological modifications above mentioned for major workers are shared with some other New World *Pheidole* species. *Pheidole aberrans* and *P. deima* are included in two distinct species groups proposed by Wilson (2003) based on external morphology. All major workers belonging to the *aberrans* group present the pro and mesonotum broadly fused and strongly curved. This peculiar shape of the promesonotum is shared, less prominently, with some members of the *tristis* group, such as *Pheidole bellatrix* Wilson, *Pheidole bison* Wilson, *Pheidole eparmata* Wilson, *Pheidole pullula* Santschi, and *Pheidole synarmata* Wilson. On the other hand, *P. deima*, as a member of *distorta* group, presents the mesonotum broadly and strongly projected. This bizarre morphological feature is markedly unique and not shared with any other *Pheidole* species. Considering the remarkable external morphology of *P. aberrans* and *P. deima*, shared with all the other members of their groups, it is expected that all species in the *aberrans* and *distorta* groups also present the same internal modifications, including the dilation on the esophagus.

Our results indicate a unique set of cuticular modifications in *Pheidole* related to the auxiliary crop, thus promoting an alternative route of storing liquid food in the colony, and not observed in other

groups with the same dilatation in the esophagus (e.g. physogastric queens and ponerine workers, as previously mentioned). Both *P. aberrans* and *P. deima* have colonies that are rarely found with few foraging workers, and majors are not often recruited out of the nest (Bruch, 1916; Wilson, 2003; ACF personal observation). Not much is known about the biology of these species, with the most extensive work for *P. aberrans* published by Bruch (1916), in which the author described the nest and worker behavior. Minor workers of *P. aberrans* can be collected under food resources (e.g. cow dung), on some occasions using superficial irregular galleries, shared with other species (Bruch, 1916). Considering *P. deima*, Wilson (2003) mentioned the possible relation of majors of this species with specialized functioning as house guards, as their head is extremely modified, presenting a deep median excavation.

Major workers of *P. aberrans* and *P. deima* may play an important role, like replete workers, as a reservoir for liquids, with the thoracic crop assuming an analogous function to the crop. In replete workers the crop is greatly distended with liquid food, with this distention reaching the point where the gastral segments separate, causing a strong stretch in the intersegmental membranes (Hölldobler and Wilson, 1990). The young replete workers start



**Fig. 3.** Micro-CT 2D sagittal section of *Pheidole aberrans* (A [major] and B [minor]) *Pheidole deima* (C [major] and D [minor]) and *Pheidole cursor* (E [major] and F [minor]) showing the esophagus and abdominal crop. Note the presence of a dilated esophagus (A–D) in comparison with a typical, unmodified esophagus (E and F).

doing their duties in the colony while their gaster is still soft and elastic (Wheeler, 1908, 1910). After ample storage of liquids in their crop, the gaster becomes so distended that these workers face difficulties in moving, thus being forced to remain permanently in the nest acting as a living reservoir (Wheeler, 1908, 1910; Hölldobler and Wilson, 1990). Extreme examples are found in arid conditions of the American Southwest (e.g. *Myrmecocystus*) and Australian deserts (e.g. *Camponotus*, *Melophorus*, and *Leptomymex*), in which replete workers provide an advantage in surviving the harshest seasons (Bartz and Hölldobler, 1982). Contrary to this pattern, species whose esophagus have a reservoir function would not suffer from locomotor impediments, thus being able to develop additional activities inside and outside the nest. Thus, *Pheidole* majors could have a potential role as repletes, not compromising its activity in defense and food processing; as well as in minors, allowing a greater capacity for liquid food transportation.

The occurrence of a thoracic crop in *P. aberrans* and *P. deima*, and the possibility of its existence in the whole *aberrans* and *distorta* group represent a new opportunity for task differentiation and the potential role of majors as repletes. Few cases of majors with distended gaster, acting as repletes, were described for *Pheidole* (Wilson, 1984; Tsuji, 1990; Lachaud et al., 1992). The existence of such specialization seems more common than expected for the genus, with several unreported cases that show a similar set of morphological modifications related to the replete workers function. Some new species with a peculiar morphology were published recently by Salata and Fisher (2020), such as *Pheidole avaratra* and *Pheidole longipilosa*. In both species, major workers (CASENTO303182 and CASENTO496936, respectively) can present a conspicuous distended gaster, probably caused by the large accumulation of liquid in the gastral crop. Additionally, *P. avaratra* and *P. longipilosa* also have modifications associated with the

mesosoma, with the pro- and mesothorax broadly fused and the promesonotum strongly convex, similar to *P. aberrans*. Some other cases of *Pheidole* species in the Old World with this notable mesosoma shape are *Pheidole cryptocera* Emery, *Pheidole laminata* Emery, *Pheidole rugofitarata* Salata and Fisher, and *Pheidole vohe-marensis* Salata and Fisher, as well as all the members of the Malagasy *makaensis* group. But it is not clear if the mesosoma shape in those species predicts the existence of a thoracic crop. Therefore, its presence may be restricted to the two South American groups.

For *aberrans* and *deima* groups, the possibility of storing liquids in the colony would allow a considerable adaptive advantage, since major workers are not often recruited to process and carry food resources. Additionally, considering the low number of minor workers of *P. aberrans* and *P. deima* found foraging, there is a limitation in the loading of resources to the nest. The possibility of the distention of the esophagus enables that foraging minors can more efficiently carry liquid resources and keep these conserved in their thoracic and gastric crops. For these species, the majors, working as live reservoirs, would make it possible to store these resources for a long period. Nevertheless, no case of food storage was reported in these species; thus, the food should be directly processed by majors and/or distributed to the brood. There are no data regarding *P. aberrans* and *P. deima* interacting with sucking insects or extrafloral nectaries, so the sources of liquid resources are not clear. But some studies (i.e. Cassill et al., 2005) suggested that the stockpiled liquid may be associated with the body fluids obtained from preys. However, more data on natural history and food preference are needed to understand the function of the thoracic crop in these species. Also, studies on colony ergonomics would be valuable to determine the putative advantages of the esophageal dilation during periods of food scarcity.

Studies on ant morphology have previously focused mainly on the external anatomy (e.g. Serna and Mackay, 2010; Keller, 2011).



With new methodologies in data acquisition and processing, mainly based on micro-computed tomography technology, the study of internal structures has become more accessible (e.g. Khalife et al., 2018; Lillico-Ouachour et al., 2018; Kubota et al., 2019; Liu et al., 2019; Richter et al., 2019). Our results indicate the importance of the internal morphology in understanding the function and consequences of modifications in unexplored structures in ants. Combining data on external and internal morphology, we may be able to explain the functional mechanisms associated with anatomical groups, as well as its consequences in the diversification and prevalence of those organisms.

## CRediT author statement

**Casadei-Ferreira:** Conceptualization, Methodology, Investigation, Writing - Original Draft, Visualization; **Fischer:** Methodology, Writing - Review & Editing; **Economio:** Resources, Writing - Review & Editing, Supervision.

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## References

- AntWeb, 2020. AntWeb Version 8.24. California Academy of Science. Online at: <https://www.antweb.org>. (Accessed 7 April 2020).
- Bartz, S.H., Hölldobler, B., 1982. Colony founding in *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae) and the evolution of foundress associations. *Behav. Ecol. Sociobiol.* 10 (2), 137–147.
- Blender, 2020. Blender – a 3D Modelling and Rendering Package. Stichting Blender Foundation, Amsterdam. Retrieved from: <http://www.blender.org>.
- Børgesen, L.W., 2000. Nutritional function of replete workers in the pharaoh's ant, *Monomorium pharaonis* (L.). *Insectes Sociaux* 47 (2), 141–146.
- Bruch, C., 1916. Contribución al estudio de las hormigas de la provincia de San Luis. *Rev. Mus. La Plata* 23, 291–357.
- Caetano, F.H., 1988. Anatomia, histologia e histoquímica do sistema digestivo e excretor de operárias de formigas (Hymenoptera, Formicidae). *Naturalia* 13, 129–174.
- Caetano, F.H., 1993. Ultramorfologia del tracto digestivo de *Neoponera villosa* (Formicidae). In: Resúmenes del XV Congreso Nacional de Entomología, 11 a 13 de Noviembre, p. 43 (Valdivia-Chile).
- Caetano, F.H., Lage Filho, A.L., 1982. Anatomia e histologia do trato digestivo de formigas do gênero *Odontomachus* (Hymenoptera, Ponerinae). *Naturalia* 7, 125–134.
- Caetano, F.H., Mathias, M.I.C., Overal, W.L., 1986. Anatomia e histologia comparada do trato digestivo de *Dinoponera gigantea* e *Paraponera clavata* (Formicidae, Ponerinae). *Naturalia* 11, 125–134.
- Caetano, F.H., Tomotake, M.E.M., Mathias, M.I.C., 1997. The role of ants esophagus in the trophallaxis (Formicidae). *Recent Adv. Ecobiol. Res.* 2, 397.
- Caetano, F.H., Tomotake, M.E.M., Pimentel, M.A.L., Mathias, M.I.C., 1990. Internal morphology of workers of *Dolichoderus attelaboides* (Fabricius, 1775) (Formicidae: Dolichoderinae). I. Digestive tract and associated excretory system. *Naturalia* 15, 57–65.
- Casadei-Ferreira, A., 2019. Pheidole. In: Starr, C. (Ed.), *Encyclopedia of Social Insect*. Springer, Cham, ISBN 978-3-319-90306-4.
- Cassill, D.L., Butler, J., Vinson, S.B., Wheeler, D.E., 2005. Cooperation during prey digestion between workers and larvae in the ant, *Pheidole spadonia*. *Insectes Sociaux* 52 (4), 339–343.
- Chapman, R.F., Chapman, R.F., 2013. *The Insects: Structure and Function*, fifth ed. Cambridge University press, Cambridge.
- Eisner, T., Brown, W.L., 1958. The evolution and social significance of the ant proventriculus. *Proc. Tenth Int. Congr. Entomol.* 2 (503), e508.
- Fischer, G., Azorsa, F., Garcia, F.H., Mikheyev, A.S., Economio, E.P., 2015. Two new phragmotic ant species from Africa: morphology and next-generation sequencing solve a caste association problem in the genus *Carebara* Westwood. *ZooKeys* (525), 77.
- Fujioka, H., Okada, Y., 2019. Liquid exchange via stomodeal trophallaxis in the ponerine ant *Diacamma* sp. from Japan. *J. Ethol.* 37 (3), 371–375.
- Glancey, B.M., Stringer Jr., C.E., Craig, C.H., Bishop, P.M., Martin, B.B., 1973. Evidence of a replete caste in the fire ant *Solenopsis invicta*. *Ann. Entomol. Soc. Am.* 66 (1), 233–234.
- Greenwald, E., Segre, E., Feinerman, O., 2015. Ant trophallactic networks: simultaneous measurement of interaction patterns and food dissemination. *Sci. Rep.* 5, 12496.
- Hanisch, P.E., Dräger, K., Yang, W.H., Tubaro, P.L., Suarez, A.V., 2020. Intra- and interspecific variation in trophic ecology of 'predatory' ants in the subfamily Ponerinae. *Ecol. Entomol.* 45 (3), 444–455.
- Hasegawa, E., 1993. Caste specialization in food storage in the dimorphic ant *Colobopsis nipponicus* (Wheeler). *Insectes Sociaux* 40 (3), 261–271.
- Heterick, B.E., Castalanelli, M., Shattuck, S.O., 2017. Revision of the ant genus *Melophorus* (Hymenoptera, Formicidae). *ZooKeys* 1 (700).
- Hölldobler, B., Wilson, E.O., 1990. *The Ants*. Harvard University Press.
- Hölldobler, B., Wilson, E.O., 2009. *The Superorganism: the Beauty, Elegance, and Strangeness of Insect Societies*. WW Norton and Company.
- Janet, C., 1907. Anatomie du corselet et histoyse des muscles vibrateurs, apres le vol nuptial, chez la reine de la fourmi (*Lasius niger*). Ducortieu et Gout, edit. Lomoges, p. 149.
- Khalife, A., Keller, R.A., Billen, J., Hita Garcia, F., Economio, E.P., Peeters, C., 2018. Skeletomuscular adaptations of head and legs of *Melissotarsus* ants for tunnelling through living wood. *Front. Zool.* 15, 30.
- Keller, R.A., 2011. A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the poneromorph subfamilies. *Bull. Am. Mus. Nat. Hist.* 2011 (355), 1–90.
- Kikinis, R., Pieper, S.D., Vosburgh, K., 2014. 3D slicer: a platform for subject-specific image analysis, visualization, and clinical support. In: Jolesz, Ferenc A. (Ed.), *Intraoperative Imaging Image-Guided Therapy*, vol. 3(19), pp. 277–289. ISBN: 978-1-4614-7656-6 (Print) 978-1-4614-7657-3.
- Kubota, H., Yoshimura, J., Niitsu, S., Shimizu, A., 2019. Morphology of the tentorium in the ant genus *Lasius* Fabricius (Hymenoptera: Formicidae). *Sci. Rep.* 9 (1), 1–8.
- Lachaud, J.P., Passera, L., Grima, A., Detrain, C., Beugnon, G., 1992. Lipid storage by major workers and starvation resistance in the ant *Pheidole pallidula* (Hymenoptera, Formicidae). In: Billen, J. (Ed.), *Biology and Evolution of Social Insects*. Leuven University Press; 1992, pp. 153–160.
- Lanan, M.C., Rodrigues, P.A.P., Agellon, A., Jansma, P., Wheeler, D.E., 2016. A bacterial filter protects and structures the gut microbiome of an insect. *ISME J.* 10 (8), 1866–1876.
- LeBoeuf, A.C., Waridel, P., Brent, C.S., Gonçalves, A.N., Menin, L., Ortiz, D., Ribagroguez, O., Koto, A., Soares, Z.G., Privman, E., Miska, E.A., 2016. Oral transfer of chemical cues, growth proteins and hormones in social insects. *Elife* 5, e20375.
- Lillico-Ouachour, A., Metscher, B., Kaji, T., Abouheif, E., 2018. Internal head morphology of minor workers and soldiers in the hyperdiverse ant genus *Pheidole*. *Can. J. Zool.* 96 (5), 383–392.
- Liu, S.P., Richter, A., Stoessel, A., Beutel, R.G., 2019. The mesosomal anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera). *Arthropod Syst. Phylogeny* 77 (1), 1–19.
- McCook, H.C., 1845. *The Honey Ants of the Garden of the Gods, and the Occident Ants of the America Plains: A Monograph of the Architecture and Habits of the Honey-Bearing Ant, Myrmecocystus Melliger, with Notes upon the Anatomy and Physiology of the Ailmentary Canal; Together with a Natural History of the Occident Harvesting Ants, or, Stonemound Builders of the American Plains*, vol. 4. Lippincott.
- Phillips Jr., S.A., Vinson, S.B., 1980. Source of the post-pharyngeal gland contents in the red imported fire ant, *Solenopsis invicta*. *Ann. Entomol. Soc. Am.* 73 (3), 257–261.
- Paul, J., Rocas, F., 2019. Comparative functional morphology of ant mouthparts and significance for liquid food intake. In: Krenn, H.W. (Ed.), *Insect Mouthparts*. Springer, Cham, pp. 335–359.
- Petersen-Braun, M., Buschinger, A., 1975. Entstehung und Funktion eines thorakalen Kropfes bei Formiciden-Königinnen. *Insectes Sociaux* 22 (1), 51–66.
- Probst, R.S., Wray, B.D., Moreau, C.S., Brandão, C.R., 2019. A phylogenetic analysis of the Dirt ants, *Basicerus* (Formicidae: Myrmecinae): inferring life histories through morphological convergence. *Insect Syst. Divers.* 3 (4), 3.
- Richter, A., Keller, R.A., Rosumek, F.B., Economio, E.P., Garcia, F.H., Beutel, R.G., 2019. The cephalic anatomy of workers of the ant species *Wasmannia affinis* (Formicidae, Hymenoptera, Insecta) and its evolutionary implications. *Arthropod Struct. Dev.* 49, 26–49.
- Rissing, S.W., 1984. Replete caste production and allometry of workers in the honey ant, *Myrmecocystus mexicanus* Wesmäl (Hymenoptera: Formicidae). *J. Kans. Entomol. Soc.* 347–350.
- Salata, S., Fisher, B.L., 2020. *Pheidole* Westwood, 1839 (Hymenoptera, Formicidae) of Madagascar – an introduction and a taxonomic revision of eleven species groups. *ZooKeys* 905, 1.

- Schultner, E., Oettler, J., Helanterä, H., 2017. The role of brood in eusocial Hymenoptera. *Q. Rev. Biol.* 92 (1), 39–78.
- Serna, F., Mackay, W., 2010. A descriptive morphology of the ant genus *Pro-cryptocerus* (Hymenoptera: Formicidae). *J. Insect Sci.* 10 (1).
- Snodgrass, R.E., 1935. Principles of Insect Morphology. Cornell University Press.
- Snodgrass, R.E., 1956. Anatomy of the Honey Bee. Cornell University Press.
- Traniello, J.F.A., 2010. *Pheidole*: sociobiology of a highly diverse genus. In: Breed, M.D., Moore, J. (Eds.), Encyclopedia of Animal Behavior, vol. I. Academic, Oxford, pp. 699–706.
- Tsuji, K., 1990. Nutrient storage in the major workers of *Pheidole ryukyuensis* (Hymenoptera: Formicidae). *Appl. Entomol. Zool.* 25 (2), 283–287.
- Vieira, A.S., Ramalho, M.O., Martins, C., Martins, V.G., Bueno, O.C., 2017. Microbial communities in different tissues of *Atta sexdens rubropilosa* leaf-cutting ants. *Curr. Microbiol.* 74 (10), 1216–1225.
- Wheeler, W.M., 1908. Honey ants, with a revision of the American Myrmecocysti. *Bull. Am. Mus. Nat. Hist.* 24 (20), 345–397.
- Wheeler, W.M., 1910. Ants: Their Structure, Development and Behavior, vol. 9. Columbia University Press.
- Wilson, E.O., 1984. The relation between caste ratios and division of labor in the ant genus *Pheidole* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* 16 (1), 89–98.
- Wilson, E.O., 2003. *Pheidole in the New World: a Dominant, Hyperdiverse Ant Genus*, vol. 1. Harvard University Press.