Evidence of a thoracic crop in workers, soldiers and queens of *Carebara perpusilla* ants (Formicidae: Myrmicinae)

Adam Khalife[1,*], Johan Billen[2] & Evan P. Economo[3]

[1] Laboratory of Entomology, Faculty of Agriculture, Kagawa University, Ikenobe, Miki, Kagawa Prefecture, 761-0795, Japan

[2] Zoological Institute, University of Leuven, 3000 Leuven, Belgium

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

* corresponding author: adam.khalife@kagawa-u.ac.jp

Abstract

The ability to share and store food is paramount in group-living animals, allowing a finely tuned distribution of resources over time and individuals and an enhanced survival over periods of food scarcity. Ants have several ways to store food: one of them is their gastral crop, also known as a ‘social stomach’. Nutrients in the crop can be regurgitated to nestmates through oral trophallaxis (mouth-to-mouth) or proceed to the midgut by opening the proventriculus, a valve connecting the crop to the midgut. However, some ants are also known to have a so-called ‘thoracic crop’, an extension of the esophagus that allows for additional storage space. In this study, we provide the first evidence of a thoracic crop in the genus *Carebara*, in reproductive (queen) and sterile (soldier and worker) castes. We discuss how the ant body plan allowed for the evolution of a novel food storage structure in the mesothorax.

Keywords: microCT, mesosomal crop, replete, major workers, *Oligomyrmex*

Introduction

Food resources vary in time and space, making food storage very important. However, storing food is challenging: light, oxygen, moisture and warm temperature are propitious to many life forms that include not only the food consumer but also bacteria, fungi or other uninvited food parasites. Even though eating food on the spot can be a suitable strategy for solitary animals, the ability to store food is desirable if not prerequisite for group-living. Mankind found creative solutions to store food even since the Neolithic, when human societies were nomadic hunter-gatherers, divided into three categories according to Morgan (2012): 1) technological, with external storage of food in designated areas, such as mobile granaries for cereals; 2) biological, *i.e.* internal storage as fat; and 3) social, through the redistribution of food resources. More sophisticated food-storing techniques emerged as societies transitioned to a sedentary lifestyle (Kuijt and Finlayson 2009) such as cooling, salting, fermentation and burial (Abdulmumeen et al. 2012). Nowadays, combined food preservatives in addition to sterilizers, refrigerators and freezers can preserve even dairy products and vegetables for days or weeks (Leistner 2000).

But millions of years before mankind, other group-living animals successfully overcame the challenge of food storage, such as the ants. Ants live in colonies and build perennial nests where tasks are shared between the reproductive caste and the worker caste. Because only part of the worker caste actually forages, ants evolved diverse ways to store and distribute food in the colony, which can be divided into two categories: 1) external, analogous to technological, with bulk storage in a particular area of the nest; and 2) internal, which is the combination of biological (individual) and social storage. The most “ancestral” strategy of external storage is to paralyze a prey by stinging and carry it back to the nest to feed the colony. This strategy is commonly observed in wasps (Steiner 1986) and poneroid ants. Paralyzed prey can be piled in the nest and stay alive until consumption for a few days to two weeks in *Harpegnathos saltator* (Maschwitz et al. 1979). Furthermore, granivorous ants (*e.g. Messor, Pogonomyrmex,* some species of *Pheidole*) store seeds in dedicated chambers analogous to human granaries (Tschinkel 1999; Johnson 2000; Reyes-López and Fernández-Haeger 2002). On the other hand, there are two main internal storage strategies in ant colonies. The first one is trophic eggs, non-viable eggs used to redistribute nutrients from an individual's fat tissue to other colony members (Gobin and Ito 2002). Queens can lay trophic eggs during colony founding to feed their first larvae (Masuko 2023). Despite being sterile, workers have retained their ovaries in most species and can hence lay trophic eggs. These eggs are morphologically distinct from haploid unfertilized eggs and can be stored to survive starvation periods (Gobin et al. 1998; Gobin and Ito 2000; Lee et al. 2022; Meurville and LeBoeuf 2021). The second internal storage strategy involves the crop, a pear-shaped bag located in the gaster (abdomen) at the posterior end of the foregut. Liquid food from the crop can either be regurgitated to nestmates through oral trophallaxis (mouth-to-mouth) or proceed to the midgut for digestion with the opening of a cuticular valve called the proventriculus (reviewed by Meurville and LeBoeuf 2021). In derived ant subfamilies, the crop is expandable, and in some species workers or soldiers can act as "repletes". Even though this term is lacking a clear definition, we call repletes individuals with a crop filled so much that it distends the gaster and intersegmental membranes become visible (Glancey et al. 1973; Burgett and Young 1974; Hasegawa 1993; Børgesen 2000; Yang 2006). In the most extreme cases such as *Myrmecocystus* (the honeypot ant), repletes can barely walk and merely act as food dispensers (Rissing 1984). An expandable crop increases the internal storage capacity per individual, which improves the colony storage ability.

In addition, Petersen-Braun and Buschinger reported in 1975 another form of internal food storage in ant queens that they called the thoracic crop. The esophagus is the intermediate part of the foregut, between the pharynx and the crop, and is typically a long and narrow tube in ants (Baroni Urbani and de Andrade 1997; Solis et al. 2009, 2013). The thoracic crop is a dilation of the esophagus within the mesosoma (thorax) that has a similar function and content as the gastral crop (Petersen-Braun and Buschinger 1975; Vander Meer et al. 1982). This structure has been identified in queens of three major subfamilies:

Ponerinae (*Neoponera, Odontomachus,* Caetano, 1988; Caetano and Tomotake, 1997), Formicinae (*Lasius*, Janet, 1907; Kurihara et al., 2022; Matte and Billen, 2021) and Myrmicinae (*Leptothorax, Monomorium, Myrmica, Solenopsis, Stenamma*, Glancey et al., 1981; Petersen-Braun and Buschinger, 1975). This phenomenon likely appeared early in the ant evolution and might be widespread, as suggested by recent evidence of an enlarged esophagus in the Cretaceous (98 Ma) ant queen *Zigrasimecia* (Zhuang et al. 2022). After mating, the histolysis of flight muscles leaves a dorsal space in the mesosoma that is quickly filled by the esophagus expansion (Kurihara et al. 2022). This thoracic crop stores the metabolized fat bodies and flight muscles that can then be regurgitated to feed the first larvae or used as energy for egg-laying. Nonetheless, subsequent investigations revealed that the thoracic crop is not a queen-specific structure. It was found in workers of Ponerinae (*Dinoponera, Neoponera, Odontomachus*, Caetano 1988; Caetano et al. 1986; Caetano and Lage Filho 1982), Paraponerinae (*Paraponera*, Caetano et al., 1986), Dolichoderinae (*Dolichoderus*, Caetano et al., 1990), Ectatomminae (*Ectatomma*, Caetano 1988) and Myrmicinae (*Pheidole*, Casadei-Ferreira et al. 2020). Most poneroid ants and Ectatomminae do not regurgitate food to their larvae (Keller and Peeters 2021), so the role of the thoracic crop in these species remains to be understood. Besides, a thoracic crop has been shown in males of *Neoponera villosa* (Ponerinae) and *Cephalotes pusillus* (Myrmicinae, Caetano 1993). Males are rarely trophallactic donors (but see Hölldobler 1966 and Hashimoto et al. 1995), so it is likely an additional space for their own energy.

In contrast, the existence of a thoracic crop in species with major workers or soldiers has received little attention. To our knowledge, it has only been studied in Neotropical *Pheidole* species (Casadei-Ferreira et al. 2020): in *P. aberrans* and *P. deima*, both workers and soldiers have a distended esophagus. The genus *Pheidole* is known to have replete soldiers (*P. hortensis*, *P. mendicula*, Wilson 1984; *P. ryukyuensis*, Tsuji 1990; *P. pallidula*, Lachaud et al. 1992; *P. morrisi*, Yang 2006). The evolution of the thoracic crop appears to be an alternative strategy to traditional repletism, maintaining a big storage capacity without hindering the soldiers' mobility (Casadei-Ferreira et al. 2020).

Soldiers exist in multiple other ant genera, including *Carebara* (Myrmicinae). This genus is distributed worldwide, mostly in tropical and subtropical regions (Azorsa and Fisher 2018) and includes 224 species characterized by extremely small workers (*e.g.* 1.0mm for *C. atoma*, personal data) and a subterranean or hypogaeic lifestyle. This genus is remarkable in its diversity of caste systems: some species have monomorphic workers with huge queens (e.g. *C. castanea*), others have small workers, larger soldiers and larger queens (*e.g. C. perpusilla*, formerly *Oligomyrmex*) while others have more than two sterile castes (*e.g. C. diversa*, formerly *Pheidologeton*). Sterile castes have completely lost ovaries in *Carebara* and cannot lay trophic eggs (Peeters and Ito 2015). Replete soldiers are known in this genus (*C. urichi*, Wilson 1986; *C. overbecki*, Moffett 1986; *C. butteli*, Fischer et al. 2015; *C. diversa*, Moffett 2019; *C. perpusilla*, Khalife and Peeters 2020) but the presence of a distended esophagus as an alternative or complementary food storage adaptation has never been investigated. In this study, using microCT, histology and natural history observations with food dyes, we provide the first evidence of a thoracic crop in workers, soldiers and queens in a *Carebara* species, *C. perpusilla*.

Materials and Methods

Ants

A polygynous colony fragment of *C. perpusilla* was collected in a riverine forest by Christian Peeters during Ant Course in Gorongosa National Park, Sofala province, Mozambique (S 19.00133 E 34.37730, ~100m elevation), in August 2016. Ants were reared in laboratory conditions in a plaster nest (285 x 285 x 90 mm) with glass-covered chambers and tunnels. Ants were fed *ad libitum* with mealworms, crickets, and occasionally cockroaches and springtails.

A red lipophilic food dye solution was prepared by mixing Sudan III dye with alimentary oil (colza and sunflower). Mealworms were dyed by injecting 0.1 mL of this solution between two central ventral sclerites, and fed to the ants 30 minutes later. Successful injection was easily assessed through the translucent cuticle of the mealworms.

Light microscopy

The mesosoma of one queen and three workers were fixed in 2% glutaraldehyde in a sodium cacodylate buffer, postfixed in 2% osmium tetroxide, and dehydrated in a graded acetone series. Tissues were embedded in Araldite resin and serial semithin sections with a thickness of 1 µm were made using a Leica EM UC6 ultramicrotome. Sections were stained with methylene blue and thionin and observed under an Olympus BX-51 microscope.

Micro-CT scanning

One worker, one soldier and one queen of *C. perpusilla* were selected randomly from within the nest shortly after colony collection, and stored in 90% ethanol until scanning. The scanning protocol is detailed in Khalife et al. (2022): micro-CT scans were performed using a Zeiss Xradia 510 Versa scanner at the Okinawa Institute of Science and Technology in 2018 operated by the Zeiss Scout-and-Scan software. Specimens were stained for at least 24 hours in iodine prior to scanning for contrast enhancement (dice-CT, Gignac et al. 2016) before being transferred to sealed pipet tips filled with 99% ethanol. Scan settings were chosen to optimize scan quality. Reconstruction of the resulting scans was performed using XMReconstructor.

3D modeling and measurements

Three-dimensional models of the ants were built using Drishti 2.7 (Limaye 2012). External views were rendered using a black-white color gradient in the Transfer Function Editor. Internal views were created by the insertion of a longitudinal Clipping Plane with a Viewport toggled on. A Viewport is a tool to visualize a slice or stack or slice included in a

Clipping Plane. Viewport thickness was 40 for the worker and 50 for the soldier and queen, and the default color gradient was used. Snapshots were assembled in figure plates using Inkscape 1.1.

In order to quantify the esophagus cross-sectional area, four Clipping Planes with a Viewport were inserted transversally, perpendicular to the esophagus: through the first neural ganglia (NG), the profurca, the second NG and the third NG. From the Viewport, esophagus height and width was measured. But because the esophagus sometimes had an irregular shape, snapshots from the Viewport were exported and loaded in ImageJ to measure the esophagus area (cross-section).

Models of the thoracic crop and gastral crop were generated with ITK-SNAP v. 3.6.0 (Yushkevich et al. 2006) using semi-automatic segmentation followed by manual corrections. Reconstructed 3D meshes were exported as .stl surfaces and loaded into Blender v. 3.0.1 (2022) for volume calculation. Thoracic and gastral crop surfaces were separated into 2 or 3 subsurfaces of simpler shape, and an icosphere was fitted to each subsurface using the Subdivision Surface and Shrinkwrap modifiers. The volume of these icospheres was used to determine the volume of thoracic and gastral crops.

Results

The esophagus of the worker, soldier and queen are shown in Fig. 1. We report the cross-sectional area (CSA) and volume of the esophagus to discuss its size (Table 1).

In the scanned worker, the esophagus was spindle-shaped and filled the mediodorsal part of the thoracic cavity (Fig. 1B and 1C). It started as a narrow tube in the prothorax (CSA = 0.2 10^3µm²). It then grew larger in the posterior prothorax (CSA = 0.9 10^3µm²). It reached its maximum width in the mesothorax (CSA = 2.2 10^3µm²), and decreased in size in the metathorax and propodeum (CSA = 0.8 10^3µm²). Three other workers were examined using histology: two showed a similar esophagus shape (Fig. 2A), but the third was thin, cylindrical and with a folded wall suggesting its potential enlargement (Fig. 2B). The esophagus volume was 0.3 10^6µm^3 for the scanned worker, compared to 12.6 10^6µm^3 for the gastral crop (42 times larger, Table 1).

In the scanned soldier, the esophagus shape was closer to a flattened spindle, with a conspicuously wrinkled membrane (Fig. 1E and 1F). Remarkably, most of the mediodorsal thoracic cavity was only coelomic fluid, without organs (similar to the worker in Fig. 2B). We estimated the cross-sectional area of the filled esophagus by adding this space to the actual area (Fig. 1E and 1F, dotted line). The anterior esophagus had a similar width to the worker (CSA = 0.2 10^3µm^2). It became much larger in the posterior prothorax (CSA = 2.1 10^3µm^2, 9.2 10^3µm^2 when filled). Similarly to the worker, it reached its maximum cross-section in the mesothorax (CSA = 2.7 10^3µm^2, 11.5 10^3µm^2 when filled) and decreased in size in the posterior mesosoma (CSA = 0.8 10^3µm^2, 1.9 10^3µm^2 when filled). The volume of the esophagus was 2.7 10^6µm^3, 5.5 10^6µm^3 (double) when filled (Table 1). In comparison, the gastral crop volume was 226.3 10^6µm^3, 41 times larger than the filled esophagus.

In the scanned queen, the esophagus was shaped as a dilated spindle, particularly wide in the mesothorax (Fig. 1H). In the anterior prothorax, it had a similar size as the worker and the soldier (CSA = 0.2 10^3µm^2). It became larger above the profurca (CSA = 2.9 10^3µm^2) and even more in the mesothorax (above the second neural ganglia: CSA = 12.3 10^3µm^2). Finally, the size of the esophagus decreased in the posterior part of the mesosoma (CSA = 2.5 10^3µm^2). Histological sections of another queen specimen showed a similar dilation of the esophagus (Fig. 2C). The volume of the esophagus of the scanned queen was 8.2 10^6µm^3 compared to 11.1 10^6µm^3 for the gastral crop (Table 1).

	Cross-sectional area (10^3µm^2)				**Volume (10^6µm^3)**		
Caste	Anterior prothorax	Above profurca	Above NG2	Above NG3	Thoracic crop (TC)	Gastral crop (GC)	GC/TC ratio
Worker	0.2	0.9	2.2	0.8	0.3	12.6	42
Soldier	0.2	2.1	2.7	0.8	2.7	226.3	83

S_{max}	0.2	9.2	11.5	1.9	5.5		41
Queen	0.2	2.9	12.3	2.5	8.2	11.1	1.4

Table 1 Thoracic crop and gastral crop measurements from microCT scans. S_{max} = soldier with maximal esophagus distension, NG2 and NG3 = second and third neural ganglia

The scanned soldier showed a flattened esophagus but a replete gastral crop (not shown, but see Khalife and Peeters 2020, figure 7). On the contrary, the scanned queen was a reproductive and did not have a replete gastral crop but showed a thoracic crop. However, feeding a *Carebara perpusilla* colony with red-dyed food highlighted the co-occurrence of a thoracic crop and a gastral crop in most soldiers as well as wingless but non-mated queens (Fig. 3). In soldiers, the colored thoracic crop is visible dorsomedially in the mesothorax, matching the empty space observed in microCT (Fig. 1E and 3B). In queens, the red color was visible in the entire mediodorsal part of the thorax, suggesting that the esophagus can be even larger than observed in the scanned queen (Fig. 1H and 3C). Furthermore, similar to the soldiers, these queens had a replete gastral crop whose volume match or even exceed that of the soldiers (Fig. 2).

Discussion

Thoracic crop and gastral crop

Our results provide the first direct evidence of a thoracic crop in the genus *Carebara*. This is also the first species known to have a thoracic crop in workers, soldiers and queens. The esophagus can be deflated in some individuals, but a wrinkled wall and free space around indicate its capacity to expand. The thoracic crop is conspicuous in queens and soldiers, where the esophagus cross-section is 50 times larger in the mesothorax than the anterior prothorax, compared to 10 times in workers. Even though such distended esophagus in all castes rationally increases the storage capacity at the scale of the colony, the volume of the thoracic crop represents less than 3% of the gastral crop for workers and soldiers. In these sterile castes, the thoracic crop appears to be a small auxiliary reservoir for short-term storage. Nevertheless, such a minor optimization is unlikely to be the sole function of the thoracic crop, and other adaptive roles are yet to be discovered. For instance, it could be involved in the modification of trophallactic fluid composition (LeBoeuf et al. 2016). In contrast, the gaster of reproductive queens is full of ovaries with little space for the gastral crop. The presence of a thoracic crop almost doubles their storage ability, which is crucial for colony founding (Kurihara et al. 2022). In laboratory conditions, *C. perpusilla* also produced winged queens that did not mate and worked as repletes for the colony, but such individuals were not seen in the original colony fragment and are possibly a laboratory artifact (Khalife and Peeters 2020).

Combining food storage strategies

A thoracic crop in soldiers has been presented as an alternative to a replete gastral crop (Casadei-Ferreira et al. 2020), permitting a similar storage ability while keeping mobility with a fine head-gaster balance (Anderson et al. 2020). Our present study brings new data that undermines this statement. First, *C. perpusilla* soldiers have a replete gastral crop and a thoracic crop, showing that the two are not mutually exclusive. Second, the storage capacity of the gastral crop is 40 times superior to the thoracic crop in workers and soldiers, demonstrating that they are not equivalent. Third, contrary to extreme repletes that have severe locomotor impairments (*e.g. Myrmecocystus*, Rissing 1984), *C. perpusilla* repletes can move, as shown during colony emigration events (Khalife and Peeters 2020). Interestingly, Wilson (1986) refers to *C. urichi* soldiers as “semi-repletes”: their gaster is considerably swollen but they are still able to perform diverse behaviors (Wilson 1986). Despite their mobility, replete soldiers are rarely seen outside the nest in *C. perpusilla*: leaving the nest is dangerous, and the death of a replete soldier would deplete the colony's food resources. Only old soldiers (as assumed by their cuticle color) with a small gaster go out to assist in foraging or defense (Khalife and Peeters 2020).

In contrast, our results show that the storage capacity of the thoracic crop is 73% in reproductive queens, meaning that the thoracic crop almost doubles their storage capacity for liquid food. A thoracic crop is likely to substantially increase food storage in ants that only have limited space available for the crop within their gaster. This includes egg-laying queens, but also species with a limited gaster elasticity (*e.g.* poneroid ants, Peeters 1997) and trophic egg layers. Remarkably, in some species, trophic eggs can be laid by queens, workers, and soldiers (*e.g. Crematogaster* subgenus *Orthocrema*, Peeters et al. 2013, F. Ito pers. comm.). Similar to *C. perpusilla*, even though soldiers are the trophic specialists, the other castes are also able to lay trophic eggs, dampening the absence of soldiers (for example at early and late colony stages). Repletism and trophic eggs are parallel strategies unlikely to occur simultaneously because they involve filling the gaster, but both can be combined with a thoracic crop.

The dorsal mesothorax as a playground for adaptation in wingless castes

In ant queens, like other flying Hymenoptera, the mesothorax is the largest thoracic segment because it includes the wing muscles (Keller et al. 2014). In contrast, workers are wingless and have a reduced mesothorax: more space is allocated to neck muscles in the prothorax and petiole muscles in the propodeum (Peeters et al. 2020). Mesothoracic musculature is exclusively ventral (intersegmental and mesocoxal muscles) and lateral (mesotrochanteral muscles), leaving an empty space in the dorsal mesothorax (Liu et al. 2019; Aibekova et al. 2022). In Ponerinae, Paraponerinae and Ectatomminae, queen-worker dimorphism is low (Peeters 1997), and the dorsal mesothorax can be used for food storage to compensate for the reduced gastral distension capacity (Caetano 1988; Caetano et al. 1993). Other genera, for example in Formicinae and Dolichoderinae have a slender hourglass-shaped mesothorax (*e.g. Cataglyphis*, *Leptomyrmex*, Peeters et al. 2020), less likely to have available space dorsally (but see Caetano 1990). However many others, for example in subfamily Myrmicinae, may have available space in the dorsal mesothorax, especially soldiers given their larger size. One way to use this space is the development of a thoracic crop, but other options exist, such as hypertrophic labial glands in *Messor* and *Melissotarsus* (AK, pers. obs.). The adaptive potential of the dorsal mesothorax in wingless castes deserves more attention.

Conclusion

Our study reveals that food storage in ant societies can simultaneously involve multiple castes (workers, soldiers, queens) and multiple strategies (thoracic crop and gastral crop). Storage capacity is 40 times superior in the gastral crop than the thoracic crop, hinting at another unknown adaptive role for the thoracic crop in repletes. Furthermore, storing nutrients in the esophagus makes the most of an available space in the dorsal mesosoma while avoiding trade-offs between food storage and oogenesis in the gaster of reproductive and trophic egg layers. The factors underlying the evolution of a particular storage strategy as well as the combination of multiple strategies in group-living organisms remain to be investigated throughout the animal kingdom.

Acknowledgements

We are grateful to the late Christian Peeters for stimulating conversations on food storage in ants and suggesting the potential interest of *Carebara perpusilla*'s esophagus. We thank the Okinawa Institute of Science and Technology Graduate University (OIST) Imaging Section for providing access to the micro-CT scanner. We also acknowledge the assistance of An Vandoren in producing the histological sections.

Declarations

CRediT authors’ contribution statement

Adam Khalife: Methodology, Investigation (microCT and behavior), Visualization, Writing - Original Draft; Johan Billen: Investigation (histology), Visualization, Writing - Review & Editing; Evan P. Economo: Supervision, Conceptualization, Resources, Writing - Review & Editing.

Funding

This work was supported by subsidy funding to OIST and Japanese Society for the Promotion of Science (JSPS) grants (KAKENHI grant-in-aid 22F22761 for AK and 17K15180 for E.P.E.).

Competing interests

The authors declare they have no competing interests.

References

Abdulmumeen HA, Risikat AN, Sururah AR (2012) Food: Its preservatives, additives and applications. International Journal of Chemical and Biochemical Sciences 36–47. https://doi.org/10.13140/2.1.1623.5208

Aibekova L, Boudinot BE, Beutel RG, et al (2022) The Skeletomuscular System of the Mesosoma of *Formica rufa* Workers (Hymenoptera: Formicidae). Insect Systematics and Diversity 6:2. https://doi.org/10.1093/isd/ixac002

Anderson PSL, Rivera MD, Suarez AV (2020) “Simple” biomechanical model for ants reveals how correlated evolution among body segments minimizes variation in center of mass as heads get larger. Integrative and Comparative Biology 60:1193–1207. https://doi.org/10.1093/icb/icaa027

Azorsa F, Fisher BL (2018) Taxonomy of the ant genus *Carebara* Westwood (Formicidae, Myrmicinae) in the Malagasy Region. ZooKeys 1–149. https://doi.org/10.3897/zookeys.767.21105

Baroni Urbani, C., & de Andrade, M. L. (1997). Pollen eating, storing, and spitting by ants. Naturwissenschaften, 84(6), 256-258.

Børgesen LW (2000) Nutritional function of replete workers in the pharaoh’s ant, *Monomorium pharaonis* (L.). Insectes Sociaux 47:141–146. https://doi.org/10.1007/PL00001692

Burgett DM, Young RG (1974) Lipid storage by honey ant repletes. Annals of the Entomological Society of America 67:743–744. https://doi.org/10.1093/aesa/67.5.743

Caetano FH (1988) Anatomia, histologia e histoquímica do sistema digestivo e excretor de operárias de formigas (Hymenoptera, Formicidae). Naturalia 13:129-174

Caetano FH (1993) Ultramorfologia del tracto digestivo de *Neoponera villosa* (Formicidae). Resumenes del XV Congreso Nacional de Entomologia (Vol. 11, p. 43)

Caetano FH, Camargo-Mathias MI, Overal WL (1986) Anatomia e histologia comparada do trato digestivo de *Dinoponera gigantea* e *Paraponera clavata* (Formicidae, Panerinae). Naturalia (São José Rio Preto) 11–12:125–134

Caetano FH, Lage Filho AL (1982) Anatomia e histologia do trato digestivo de formigas do gênero *Odontomachus* (Hymenoptera, Ponerinae). Naturalia (São José do Rio Preto) 7:125–134

Caetano FH, Tomotake MEM (1997) The Role of Ants Esophagus in the Trophallaxis. Recent Advances in Ecobiological Research 2:397

Caetano FH, Tomotake MEM, Pimentel MAL, Mathias MIC (1990) Internal morphology of workers of *Dolichoderus attelaboides* (Fabricius, 1775) (Formicidae: Dolichoderinae). I. Digestive tract and associated excretory system. Naturalia (São Paulo) 15:57–65

Casadei-Ferreira A, Fischer G, Economo EP (2020) Evidence for a thoracic crop in the workers of some Neotropical *Pheidole* species (Formicidae: Myrmicinae). Arthropod Structure & Development 59:100977. https://doi.org/10.1016/j.asd.2020.100977

Fischer G, Azorsa F, Hita Garcia F, et al (2015) Two new phragmotic ant species from Africa: morphology and next-generation sequencing solve a caste association problem in the genus *Carebara* Westwood. ZooKeys 525:77–105. https://doi.org/10.3897/zookeys.525.6057

Gignac PM, Kley NJ, Clarke JA, et al (2016) Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. Journal of Anatomy 228:889–909. https://doi.org/10.1111/joa.12449

Glancey BM, Glover A, Lofgren CS (1981) Thoracic crop formation following dealation by virgin females of two species of *Solenopsis*. Florida Entomologist 64:454

Glancey BM, Stringer CE Jr, Craig CH, et al (1973) Evidence of a replete caste in the fire ant *Solenopsis invicta*. Annals of the Entomological Society of America 66:233–234. https://doi.org/10.1093/aesa/66.1.233

Gobin, B., Peeters, C., & Billen, J. (1998). Production of trophic eggs by virgin workers in the ponerine ant *Gnamptogenys menadensis*. Physiological Entomology 23(4):329-336

Gobin, B., & Ito, F. (2000). Queens and major workers of *Acanthomyrmex ferox* redistribute nutrients with trophic eggs. Naturwissenschaften 87:323-326

Hasegawa E (1993) Caste specialization in food storage in the dimorphic ant *Colobopsis* *nipponicus* (Wheeler). Insectes Sociaux 40:261–271. https://doi.org/10.1007/BF01242362

Hashimoto, Y., Yamauchi, K., & Hasegawa, E. (1995). Unique habits of stomodeal trophallaxis in the ponerine ant *Hypoponera* sp. Insectes Sociaux 42:137-144

Hölldobler, B. (1966). Futterverteilung durch Männchen im Ameisenstaat. Zeitschrift für vergleichende Physiologie 52:430-455

Janet C (1907) Anatomie du Corselet et Histolyse des Muscles Vibrateurs, Après le Vol Nuptial chez la Reine de la Fourmi: *Lasius niger*. Vol. 1. Ducourtieux et Gout

Johnson RA (2000) Seed-harvester ants (Hymenoptera: Formicidae) of North America: an overview of ecology and biogeography. Sociobiology 36:89–122

Keller RA, Peeters C, Beldade P (2014) Evolution of thorax architecture in ant castes highlights trade-off between flight and ground behaviors. eLife 3:e01539. https://doi.org/10.7554/eLife.01539

Keller, R. A., & Peeters, C. (2021). Poneroid ants. In Encyclopedia of Social Insects (pp. 749-754). Cham: Springer International Publishing

Khalife A, Peeters C (2020) Food storage and morphological divergence between worker and soldier castes in a subterranean myrmicine ant, *Carebara perpusilla*. Journal of Natural History 54:3131–3148. https://doi.org/10.1080/00222933.2021.1890851

Khalife A, Peeters C, Economo EP (2022) Minute workers and large soldiers in the subterranean ant *Carebara perpusilla*: Musculoskeletal consequences of Haller’s rule in the thorax. Arthropod Structure & Development 69:101188. https://doi.org/10.1016/j.asd.2022.101188

Kuijt I, Finlayson B (2009) Evidence for food storage and predomestication granaries 11,000 years ago in the Jordan Valley. Proceedings of the National Academy of Sciences

106:10966–10970. https://doi.org/10.1073/pnas.0812764106

Kurihara Y, Ogawa K, Chiba Y, et al (2022) Thoracic crop formation is spatiotemporally coordinated with flight muscle histolysis during claustral colony foundation in *Lasius japonicus* queens. Arthropod Structure & Development 69:101169. https://doi.org/10.1016/j.asd.2022.101169

Lachaud JP, Passera L, Grimal A, et al (1992) Lipid storage by major workers and starvation resistance in the ant *Pheidole pallidula* (Hymenoptera, Formicidae). In First European Congress of Social Insects, Leuven (Belgium), 19-22 Aug 1991. Leuven University Press

LeBoeuf, A. C., Waridel, P., Brent, C. S., Gonçalves, A. N., Menin, L., Ortiz, D., ... & Keller, L. (2016). Oral transfer of chemical cues, growth proteins and hormones in social insects. eLife 5:e20375. https://doi.org/10.7554/eLife.20375.001

Lee, C. C., Lin, Y. T., Chiu, M. C., Yang, C. C. S., & Lin, C. C. (2022). Preferential food allocation to an essential worker subcaste in the invasive yellow crazy ant, *Anoplolepis gracilipes* (Hymenoptera: Formicidae). Myrmecological News, 32:139-147. https://doi.org/10.25849/myrmecol.news_032:139

Leistner L (2000) Basic aspects of food preservation by hurdle technology. International Journal of Food Microbiology 55:181–186. https://doi.org/10.1016/S0168-1605(00)00161-6

Limaye A (2012) Drishti: a volume exploration and presentation tool. In: Developments in X-Ray Tomography VIII. SPIE, pp 191–199.

Liu S-P, Richter A, Stoessel A, Beutel RG (2019) The mesosomal anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera). Arthropod Systematics & Phylogeny 77:1-19. https://doi.org/10.26049/ASP77-1-2019-01

Maschwitz U, Hahn M, Schönegge P (1979) Paralysis of prey in ponerine ants. Naturwissenschaften 66:213–214. https://doi.org/10.1007/BF00366035

Matte A, Billen J (2021) Flight muscle histolysis in *Lasius niger* queens. Asian Myrmecology 13. https://doi.org/10.20362/AM.013003

Meurville M-P, LeBoeuf AC (2021) Trophallaxis: the functions and evolution of social fluid exchange in ant colonies (Hymenoptera: Formicidae). Myrmecological News, 31. https://doi.org/10.25849/MYRMECOL.NEWS_031:001

Moffett MW (1986) Notes on the Behavior of the Dimorphic Ant *Oligomyrmex overbecki* (Hymenoptera: Formicidae). Psyche: A Journal of Entomology 93:107–116. https://doi.org/10.1155/1986/62438

Moffett MW (2019) Marauder Ants (*Carebara* in Part). In: Starr C (ed) Encyclopedia of Social Insects. Springer International Publishing, Cham, pp 1–8 https://doi.org/10.1007/978-3-319-90306-4_75-1

Morgan C (2012) Modeling Modes of Hunter-Gatherer Food Storage. American Antiquity 77:714–736. https://doi.org/10.7183/0002-7316.77.4.714

Peeters C (1997) Morphologically ‘primitive’ ants: comparative review of social characters, and the importance of queen–worker dimorphism. In: Crespi BJ, Choe JC (eds) The Evolution of Social Behaviour in Insects and Arachnids. Cambridge University Press, Cambridge, pp 372–391

Peeters, C., & Ito, F. (2015). Wingless and dwarf workers underlie the ecological success of ants (Hymenoptera: Formicidae). Myrmecological News 21:117-130

Peeters C, Keller RA, Khalife A, et al (2020) The loss of flight in ant workers enabled an evolutionary redesign of the thorax for ground labour. Frontiers in Zoology 17:33. https://doi.org/10.1186/s12983-020-00375-9

Peeters C, Lin C-C, Quinet Y, et al (2013) Evolution of a soldier caste specialized to lay unfertilized eggs in the ant genus *Crematogaster* (subgenus *Orthocrema*). Arthropod Structure & Development 42:257–264. https://doi.org/10.1016/j.asd.2013.02.003

Petersen-Braun M, Buschinger A (1975) Entstehung und Funktion eines thorakalen Kropfes bei Formiciden-Königinnen. Insectes Sociaux 22:51–66. https://doi.org/10.1007/BF02223123

Reyes-López JL, Fernández-Haeger J (2002) Food storage in the nest and seed selectivity in the harvester ant *Messor barbarus*. Sociobiology 39:1-6

Rissing SW (1984) Replete caste production and allometry of workers in the honey ant, *Myrmecocystus mexicanus* Wesmael (Hymenoptera: Formicidae). Journal of the Kansas Entomological Society 57:347–350

Solis, D. R., Caetano, F. H., Yabuki, A. T., Moretti, T. C., & Bueno, O. C. (2009). Ultramorphology of the digestive tract of *Paratrechina longicornis* (Hymenoptera, Formicidae). Sociobiology 53(1):51-59

Solis, D. R., Rossi, M. L., Fox, E. G. P., de Lima Nogueira, N., Tanaka, F. A. O., & Bueno, O. C. (2013). On the morphology of the digestive system of two *Monomorium* ant species. Journal of Insect Science 13(1):70

Steiner AL (1986) Stinging behaviour of solitary wasps. In: Piek T. (ed) Venoms of the Hymenoptera. Biochemical, pharmacological and behavioural aspects. London: Academic Press, pp 63–148

Tschinkel WR (1999) Sociometry and Sociogenesis of Colony-Level Attributes of the Florida Harvester Ant (Hymenoptera: Formicidae). Annals of the Entomological Society of America 92:80–89. https://doi.org/10.1093/aesa/92.1.80

Tsuji K (1990) Nutrient Storage in the Major Workers of *Pheidole ryukyuensis* (Hymenoptera :

Formicidae). Applied Entomology and Zoology 25:283–287. https://doi.org/10.1303/aez.25.283

Vander Meer RK, Glancey BM, Lofgren CS (1982) Biochemical changes in the crop, oesophagus and postpharyngeal gland of colony-founding red imported fire ant queens (*Solenopsis invicta*). Insect Biochemistry 12:123–127. https://doi.org/10.1016/0020-1790(82)90079-8

Wilson EO (1986) Caste and division of labor in *Erebomyrma*, a Genus of dimorphic ants (Hymenoptera: Formicidae: Myrmicinae). Insectes Sociaux 33:59–69. https://doi.org/10.1007/BF02224035

Wilson EO (1984) The relation between caste ratios and division of labor in the ant genus *Pheidole* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 16:89–98. https://doi.org/10.1007/BF00293108

Yang AS (2006) Seasonality, division of labor, and dynamics of colony-level nutrient storage in the ant *Pheidole morrisi*. Insectes Sociaux 53:456–462. https://doi.org/10.1007/s00040-005-0896-3

Yushkevich, P. A., Piven, J., Hazlett, H. C., Smith, R. G., Ho, S., Gee, J. C., & Gerig, G. (2006). User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. Neuroimage 31(3):1116-1128

Zhuang Y, Xu W, Zhang G, et al (2022) Unparalleled details of soft tissues in a Cretaceous ant. BMC Ecology and Evolution 22:146. https://doi.org/10.1186/s12862-022-02099-2

Figures

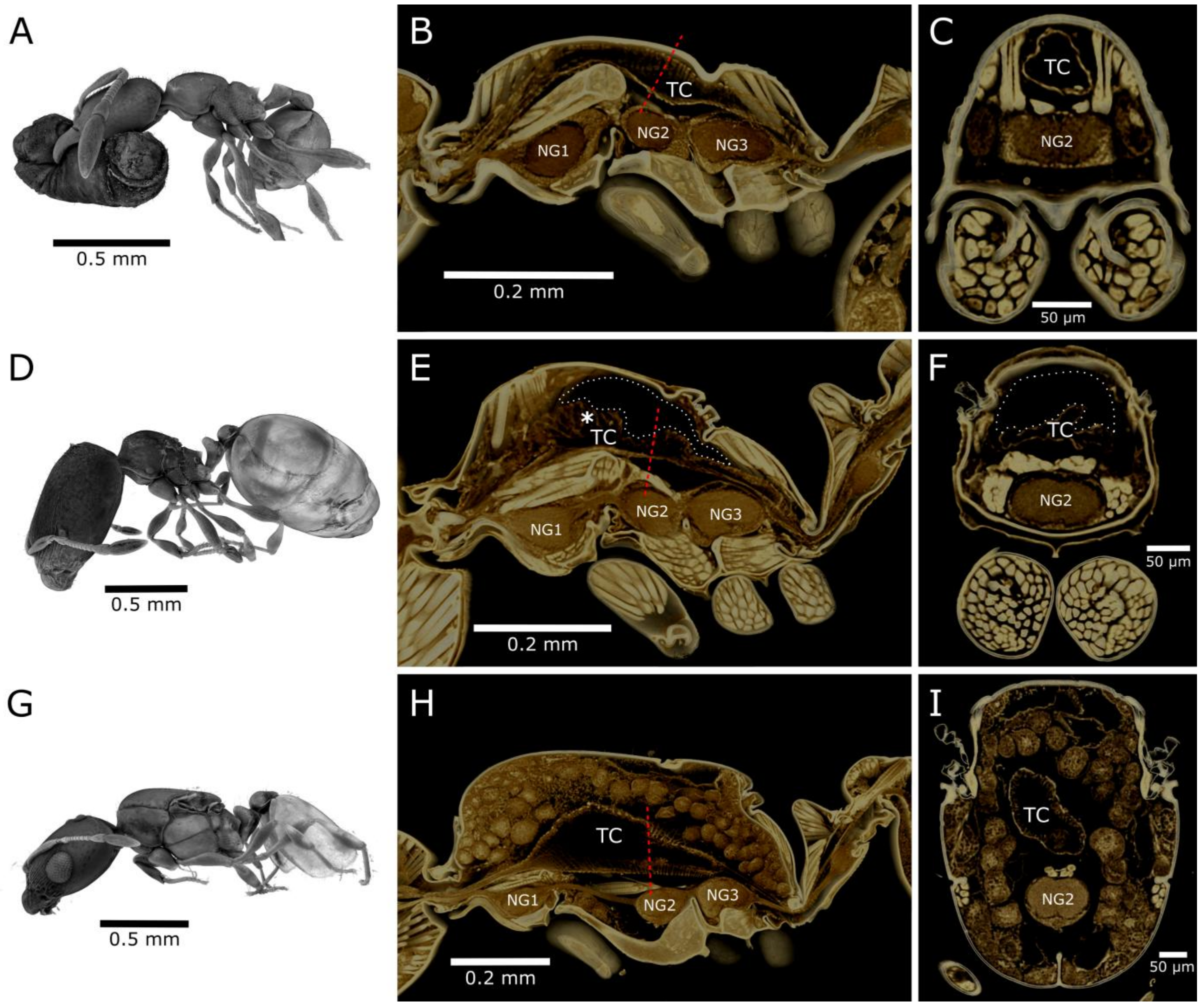

Fig. 1 3D models of *C. perpusilla* worker (A, B, C), soldier (D, E, F) and queen (G, H, I). External whole-body views (A, D, G) are matched with longitudinal (B, E, H) and transversal (C, F, I) internal views of the mesosoma. TC = thoracic crop, NG = neural ganglia. The asterisk (E) indicates a wrinkled membrane, suggesting a deflated state of the esophagus in the scanned soldier. The potential additional esophagus volume is highlighted with a dotted line (E, F)

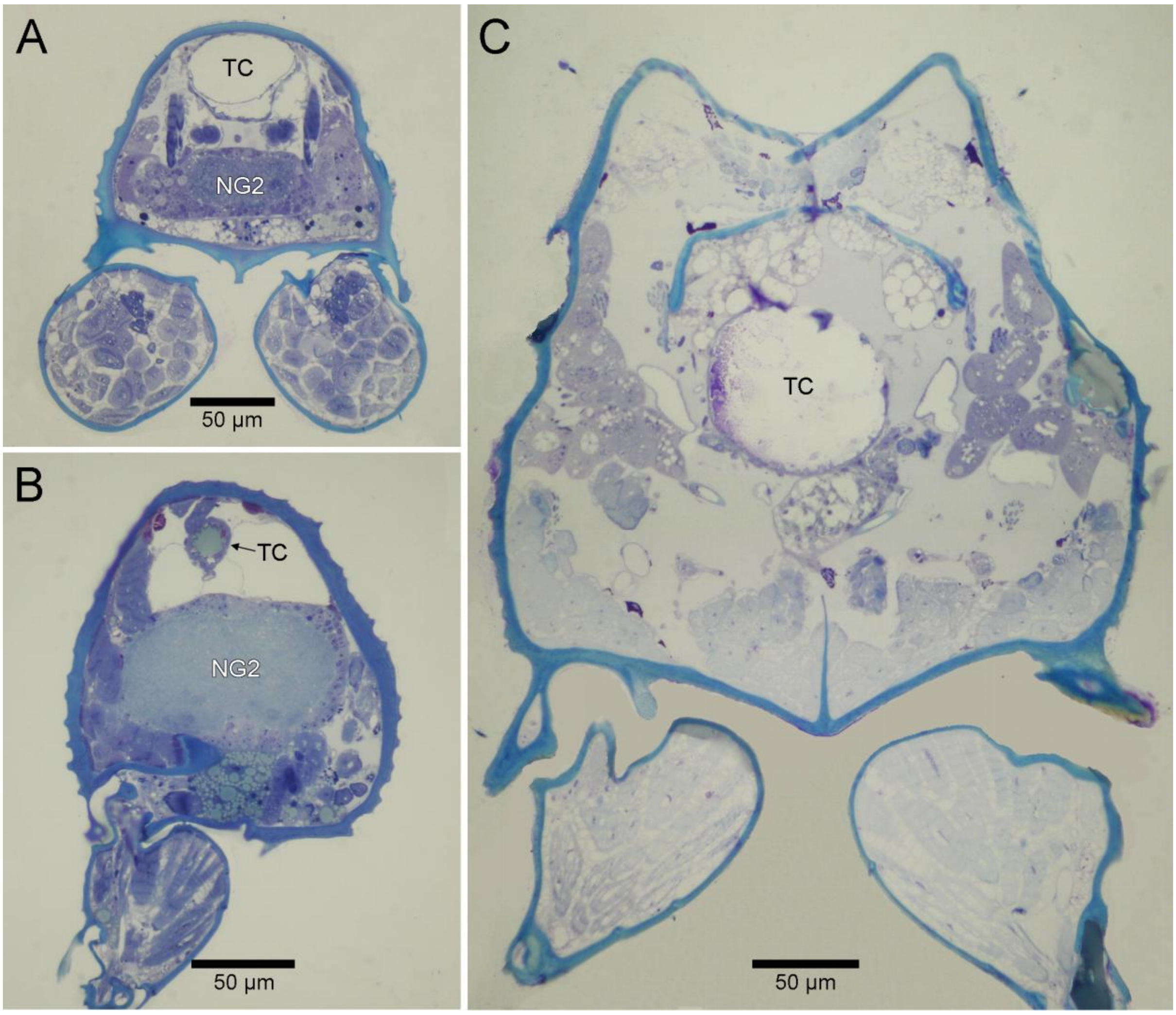

Fig. 2 Semithin cross sections through the central part of the mesosoma of *C. perpusilla*. The esophagus is extended to a thoracic crop (TC) in the worker (A) and queen (C). The esophagus may also occur deflated, but then shows a considerably wrinkled wall (worker in B). NG2 = second neural ganglia

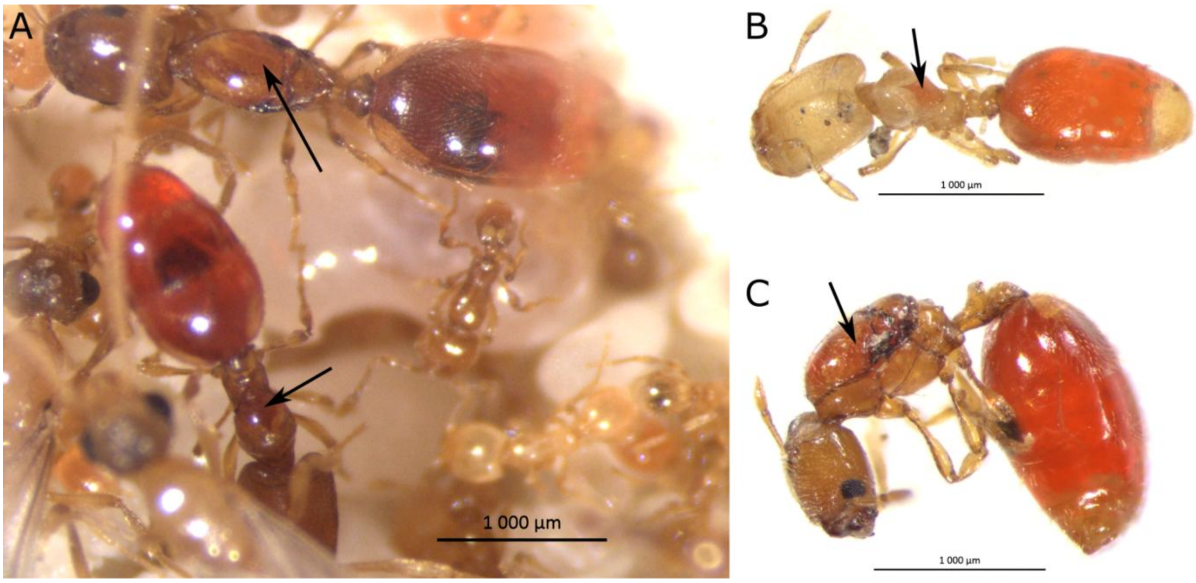

Fig. 3 Colony of *C. perpusilla* after feeding on a red-dyed mealworm (A), and individual view of soldier (B) and queen (C). Arrows indicate the thoracic crop, visible in red through the cuticle. The red color was not conspicuous in workers