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

A. Casadei-Ferreira a, *, G. Fischer b, E.P. Economob

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

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

**Article info**

- **Article history:**
  - Received 28 May 2020
  - Accepted 21 July 2020
  - Available online xxx

**Keywords:**
- Ants
- Dimorphism
- Mesosomal crop
- Liquid food
- Species group

**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.

© 2020 Elsevier Ltd. All rights reserved.

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). 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; Schultzner 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 constricted 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; Börgesen, 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...
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 stomodaeum 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 developing 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 Busching (1975) in physo gastric queens of Myrmicinae (Monomorium pharaonis [Linnaeus]) Myrmica rubra Nylander, Lepothorax unifasciatus [Latreille], Lepothorax interruptus [Schenck], Lepothorax affinis Mayr, and Stenamma westwoodi 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 attelaboloides [Fabricius]; Caetano et al., 1990), Ectatomminae (Ectatomma quadridens [Fabricius]; Caetano et al., 1988), Para ponerinae (Paraponera clavata [Fabricius]; Caetano et al., 1986) and Ponerinae (Dinoponera gigantea [Perty], Neoponera villosa [Fabricius], Neoponera obscuricornis [Emery], Odontomachus baeri [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 shelves pusillus (Santschi).

In physo gastric queens of M. pharaonis, the dilatation of the esophagus is associated with ovarian overdevelopment, due to which only little space is available to the distention of the gastric crop, thus allowing the storage of liquids in the mesosoma which only little space is available to the distention of the gaster (Santschi). For instance the proventriculus, the structure controlling liquid flow between the crop and the gaster, where the most important parts are situated (e.g. crop, proventriculus and hindgut [proctodeum]) (Wheeler, 1910).

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/μCT 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× 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 (X) × 992 × 999 (H × W × D) pixels, while voxel sizes range between 2.25 μm and 5.39 μm. 3D reconstruction of the resulting scans was done with XMReconstructor.

The 3D surface models, as well as the snapshots from the rendered slices, were generated with 3D Slicer v. 4.110 (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).](image-url)
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 the *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 function 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 gastric segments separate, causing a strong stretch in the intersegmental membranes (Holldobler and Wilson, 1990). The young replete workers start
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 Lepptomynex), 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 (CASENT0303182 and CASENT0496936, respectively) can present a conspicuous distended gaster, probably caused by the large accumulation of liquid in the gastric 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 cryptocerus Emery, Pheidole laminata Emery, Pheidole rugofarata 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 extra-floral 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; Economo: Resources, Writing - Review & Editing, Supervision.

Acknowledgments

Thanks to Rogerio Silva and Tony Peterson for sending specimens of P. cursor. Specimens of P. deim us were acquired during AntCourse/2018, we are grateful to the organizers, Nouragues Natural Reserve for allowing sampling in the protected area, Nouragues Research Station and CNRS Guyane for logistical support, and for the financial support provided by the Investissement d'Avenir grant of the Agence Nationale de la Recherche (CEBA, ANR-10-LABX-25-01). The authors thank the OIST Imaging section for access to the CT scanner. Christian Peeters, Rodrigo Feitosa, Thiago Silva, and two anonymous reviewers provided valuable comments and suggestions for previous versions of this manuscript. ACF was funded by the Brazilian Council of Research and Scientific Development [CNPq grant 140260/2016-1 and CAPES Foundation [PDSE grant 88881.189085/2018-01]. This paper was funded by the subsidy funding to OIST and JSPE Kakenhi Grant-in-Aid [No. 17K15180].

References


Caetano, F.H., 1997. The role of ants esophagus in function and consequences of modifications in unexplored struc-
tures 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.


Caetano, F.H., Tomotake, M.E.M., Pimentel, M.A.L., Mathias, M.I.C., 1997. The role of ants esophagus in function and consequences of modifications in unexplored struc-
tures 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.