Micro-CT visualisation of the vestigial alimentary canal of adult oestrid flies

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Medical and Veterinary Entomology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>MVE-17-1728.R1</td>
</tr>
<tr>
<td>Wiley - Manuscript type:</td>
<td>Short Communication</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>n/a</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Martín-Vega, Daniel; Natural History Museum, Life Sciences; University of Alcalá, Life Sciences Garbout, Amin; Natural History Museum, Imaging and Analysis Centre Ahmed, Farah; Natural History Museum, Imaging and Analysis Centre Ferrer, Luis Miguel; University of Zaragoza, Animal Pathology Lucientes, Javier; Departamento de Patología Animal, Departamento de Patología Animal Colwell, Douglas; Lethbridge Research Centre, Hall, Martin; Natural History Museum, Entomology</td>
</tr>
<tr>
<td>Category:</td>
<td>Veterinary</td>
</tr>
<tr>
<td>Abstract:</td>
<td>Oestrid flies (Diptera: Oestridae) do not feed during the adult stage as they acquire all the necessary nutrients during their parasitic larval stage. The adult mouthparts and digestive tract are therefore frequently vestigial; however, morphological data on the alimentary canal of adult oestrid flies are scarce and there is a lack of a proper visualisation of this organ system within the adult body. Here we visualise the morphology of the alimentary canal of adults of two oestrid species: Oestrus ovis L. and Hypoderma lineatum (de Villiers) with the use of non-invasive micro-computed tomography (micro-CT) and we compare it with the highly developed alimentary canal of the blow fly Calliphora vicina Robineau-Desvoidy (Diptera: Calliphoridae). Both O. ovis and H. lineatum adults showed a significant reduction of the cardia and the diameter of the digestive tract, an absence of the typical helicoidal portion of the midgut of other muscoid flies, and a lack of crop and salivary glands. Given the current interest of the alimentary canal of adult dipterans in biomedical and developmental biology studies, a further understanding of the morphology and development of this organ system in adult oestrids may provide valuable new insights into several areas of research.</td>
</tr>
</tbody>
</table>
Micro-CT visualisation of the vestigial alimentary canal of adult oestrid flies

D. Martín-Vega\textsuperscript{1,2}, A. Garbout\textsuperscript{3}, F. Ahmed\textsuperscript{3}, L. M. Ferrer\textsuperscript{4}, J. Lucientes\textsuperscript{4}, D. D. Colwell\textsuperscript{5}, M. J. R. Hall\textsuperscript{1}

\textsuperscript{1}Department of Life Sciences, Natural History Museum, London, U.K., \textsuperscript{2}Department of Life Sciences, University of Alcalá, Alcalá de Henares, Spain, \textsuperscript{3}Imaging and Analysis Centre, Natural History Museum, London, U.K., \textsuperscript{4}Department of Animal Pathology, University of Zaragoza, Zaragoza, Spain, \textsuperscript{5}Agriculture and Agri-Food Canada, Lethbridge, Canada

**Running head:** The alimentary canal of adult oestrids

Correspondence: Daniel Martín-Vega, Departamento de Ciencias de la Vida, Facultad de Ciencias, Universidad de Alcalá, Ctra. Madrid-Barcelona, km 33.6, 28805 Alcalá de Henares, Madrid, Spain. Tel.: +34918854972; E-mail: d.martin-vega@nhm.ac.uk; daniel.martinve@uah.es
Abstract. Oestrid flies (Diptera: Oestridae) do not feed during the adult stage as they acquire all the necessary nutrients during their parasitic larval stage. The adult mouthparts and digestive tract are therefore frequently vestigial; however, morphological data on the alimentary canal of adult oestrid flies are scarce and there is a lack of a proper visualisation of this organ system within the adult body. Here we visualise the morphology of the alimentary canal of adults of two oestrid species: *Oestrus ovis* L. and *Hypoderma lineatum* (de Villiers) with the use of non-invasive micro-computed tomography (micro-CT) and we compare it with the highly developed alimentary canal of the blow fly *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae). Both *O. ovis* and *H. lineatum* adults showed a significant reduction of the cardia and the diameter of the digestive tract, an absence of the typical helicoidal portion of the midgut of other muscoid flies, and a lack of crop and salivary glands. Given the current interest of the alimentary canal of adult dipterans in biomedical and developmental biology studies, a further understanding of the morphology and development of this organ system in adult oestrids may provide valuable new insights into several areas of research.

Key words. *Calliphora vicina*, *Hypoderma lineatum*, *Oestrus ovis*, cardia, crop, insect anatomy, insect gut, micro-computed tomography, organ development, salivary glands.

During their larval stage, oestrid flies are obligate parasites of vertebrates, feeding on the living tissues of the host and producing the disease condition myiasis (Hall & Wall, 1995). Whether they infest wild or domestic animals, oestrid flies have a major negative impact on both animal health and the economics of livestock production and wildlife management, with many species showing wide geographical distributions (Colwell et al., 2006). For example, the nasal bot fly, *Oestrus ovis*, is a cosmopolitan species that typically parasitises sheep and goats, although it can occasionally infest other mammals, including humans (Colwell et al., 2006). Similarly, species within the genus *Hypoderma* Latreille, commonly called warble flies, cause annual losses of millions of dollars to the cattle industry across the Holarctic region, but rarely they can rarely infest humans as well (Colwell et al., 2006). Moreover, some oestrid species may be currently expanding their host range and could potentially represent emerging infectious diseases in particular areas (Panadero et al., 2017).
Unlike other muscoid flies, oestrids do not feed on protein during the adult stage and all the necessary nutrients, including those required for reproduction, are accumulated during the long, parasitic larval stage (Anderson, 2006). The adult mouthparts are indeed frequently vestigial and non-functional in both sexes: in many genera, such as Oestrus L. or Hypoderma, the proboscis is reduced to a minute protuberance with no external opening (Wood, 2006). Apparently, the adult alimentary canal is also reduced in relation to that of other muscoid flies (Singh & Judd, 1966). However, available data on the morphology of this organ system in adult oestrids are scarce and generally limited to brief mentions highlighting the lack of certain structures in certain genera — e.g. the lack of a crop in Cephenemyia Latreille (Catts & García, 1963) — without further detail or illustration. A few more details accompanied by sketchy illustrations of the adult alimentary canal of Cephenemyia apiaca (Bennet & Sabrosky), Cuterebra latifrons (Coquillet) and Hypoderma lineatum were provided by Singh & Judd (1966) but, to the best of our knowledge, this organ system had never been imaged in full within the adult body in oestrid flies. The alimentary canal of dipterans historically had been indeed historically neglected, but it is receiving increasing attention, especially in the fruit fly Drosophila melanogaster Meigen (Diptera: Drosophilidae), due to its role as a major regulator of multiple biological processes (Leimatre & Miguel-Aliaga, 2013). The morphology of the alimentary canal of D. melanogaster had been described by Miller (1950) among others; nevertheless, the most comprehensive anatomical study of the adult alimentary canal in a dipteran is probably that of Graham-Smith (1934) on the blow fly Calliphora vicina. Also using Ca. vicina as a model organism, Martín-Vega et al. (2017) suggested that the adult digestive tract might play an essential role in maintaining a constant body volume during the histolysis and histogenesis periods of blow fly metamorphosis. Therefore, considering the significance of this organ system for dipterans, a further understanding of the morphology of the vestigial alimentary canal of adult oestrids should be of interest for many different areas of research in developmental biology.

The aim of the present exploratory study was to visualise, for the first time, the general morphology of the alimentary canal of adult O. ovis using X-ray micro-computed tomography (micro-CT). In recent years, micro-CT has become an essential tool in morphological studies as it enables, in contrast to histology and other traditional
techniques, the rapid, non-invasive and high-quality visualisation of internal structures (Martín-Vega et al., 2017). Two *O. ovis* adults (one male and one female) were obtained from third-instar larvae collected on infested sheep in the province of Zaragoza (Spain), killed and preserved by immersion in 80 % ethanol within the first day after emergence from the puparium. Each specimen was stained at the Natural History Museum, London (UK) by immersion in 0.5 M iodine in aqueous solution for two weeks and then washed and stored in 70 % ethanol for 24 hours before scanning (Martín-Vega et al., 2017). Prior to staining, the legs of the specimens were removed to enhance the penetration of the staining solution. For scanning, each specimen was placed in an Eppendorf tube containing 70 % ethanol. Each Eppendorf tube was mounted on a metal platform and scanned in a Zeiss Versa 520 system with 4x optical magnification, using a Zeiss proprietary LE6 filter and exposure set to 6 secs, current to 84 µA and voltage to 60 kV. The resulting projections were reconstructed as TIFF stacks with a voxel size of 10.5–12.5 µm³ using Zeiss proprietary software, Scout-and-Scan Control System and Reconstructor. Reconstructed data were imported into VG Studio Max 2.2 (Volume Graphics GmbH, Heidelberg, Germany), where slice stacks were rendered, reoriented and visualised in the three principal planes (cross, horizontal and sagittal). The reconstructed data were also loaded into Avizo 9.2. (Visualization Sciences Group, Bordeaux, France) for 3D rendering, segmentation and visualisation.

For comparison, three *C. vicina* adults (one male and two females) were stained, mounted and scanned replicating the same procedure described above. The *C. vicina* specimens had been reared in a laboratory colony at the Natural History Museum, London (UK) as described in Martín-Vega et al. (2017). The adult alimentary canal of *C. vicina* is fully developed when an individual emerges from the puparium but the crop is empty and deflated (Martín-Vega et al., 2017). Therefore, in order to visualise the crop fully expanded and filled with food, the male and one of the two females were collected 4 days after emergence from the puparium and had been fed with pig blood on the day of collection in order to visualise the crop expanded and filled with food. The remaining female had also been fed with pig blood but it was collected 10 days after emergence from the puparium, after ensuring that it was a gravid individual. Additionally, two *H. lineatum* adults (one male and one female), reared from third-instar larvae collected from artificially infested cattle in Lethbridge (Canada), collected 2 days after emergence from the puparium and preserved in 80 % ethanol, were also
scanned following the aforementioned protocol for further comparison and to corroborate the observations of Singh & Judd (1966).

The resulting scans revealed a greatly reduced alimentary canal in both adult *O. ovis* (Fig. 1A–E, 1L, 2A, 2C) and *H. lineatum* (1K), in contrast to the high development of this organ system in adult *Ca. vicina* (Fig. 1F–J, 1L, 2B, 2D). Compared to *Ca. vicina* and other muscoid flies, the alimentary canal of both *O. ovis* and *H. lineatum* showed a series of characteristics in concordance with the description of Singh & Judd (1966) for *H. lineatum*, with no differences between sexes: reduction of the cardia and the diameter of the digestive tract, absence of an helicoidal portion of the midgut in the abdomen, and lack of crop and salivary glands (Fig. 1A–E, 1K, 2A, 2C). The salivary glands and the crop are present in most dipterans and the latter is mainly used as the storage reservoir for proteinaceous diets destined for reproduction, although it also affects several other physiological and behavioural functions (Stoffolano & Haselton, 2013). In most dipterans, such a large quantity of nutrients can be stored in the expanded crop (Fig. 1H, 1L) that one full meal is enough for a female to produce a normal compliment of eggs (Fig. 1I) (Stoffolano & Haselton, 2013). Partial digestion of those nutrients is usually initiated in the crop, largely due to the action of salivary gland enzymes (Stoffolano & Haselton, 2013). In adult oestruids, lack of crop and salivary glands had been observed by Singh & Judd (1966) not only in *H. lineatum* but also in *Ce. apicata*; however, Catts & García (1963) described a pair of salivary glands in dissected adults of the latter species. As opposed to most oestruids, the mouthparts of *Cephenemyia* adults seem to be functional (yet underdeveloped) as they have been observed drinking water, although no feeding has been reported (Catts & García, 1963). On the other hand, the alimentary canal of *Cu. latifrons* adults is relatively well developed, with both the crop and salivary glands being present (Singh & Judd, 1966). Interestingly, *Cuterebra* females emerge with immature eggs, but they do not need to feed to mature them — like in *Cephenemyia, Cuterebra* adults have been observed drinking water under laboratory conditions, but not to consume any food provided (Anderson, 2006). Taking into account the digestive role of salivary enzymes, it is unclear if the salivary glands are functional when present in some oestruids. Whatever the case may be, in *O. ovis, H. lineatum* and most oestruid species, adult flies emerge ready to mate, with females carrying fully developed eggs in the ovaries (Fig. 2C) (Anderson, 2006). The abdomen of newly emerged oestruid flies is therefore mostly
occupied with the already mature gonads, along with fat bodies and air sacs, whereas
the diameter of the midgut is greatly reduced (Fig. 1C–D, 1K). The general reduction of
the diameter of the midgut of adult oestrids is also significant in the thoracic region
(Fig. 1A–B, 1K, 2A, 2C). Also greatly reduced is the cardia (Fig. 1A, 1K, 2A, 2C),
otherwise typically conspicuous in muscoid flies (Fig. 1F, 1L, 2B, 2D) (King, 1991).
This organ, sometimes also called “proventriculus”, contains specialised midgut cells
that secrete a complex peritrophic membrane (King, 1991); however, those secretory
cells are not well developed in Ce. apicata and Cu. latifrons and are absent in H.
lineatum, according to Singh & Judd (1966).

The adult alimentary canal is one of the largest organ systems within the body of
muscoid flies, and one of the structures showing the greatestmost drastic changes in
morphology during metamorphosis (Leimatre & Miguel-Aliaga, 2013; Martín-Vega et
al., 2017). During the pupal stage and the later development of a typical muscoid
pharate adult within the puparium, the digestive tract undergoes significant changes in
shape and volume, with the midgut progressively narrowing and becoming helicoidal in
its most distal portion (Martín-Vega et al., 2017). At the end of the intra-puparial
period, i.e. prior to emergence from the puparium, all the components of the adult
alimentary canal are fully developed (Martín-Vega et al., 2017). However, Cepeda-
Palacios & Scholl (2000) noticed that, during the intra-puparial development of adult O.
ovis, the mouthparts appear transitorily but then regress, so it would be interesting to
determine if different organs in the alimentary canal either stop their development at
some point or if they further develop but then degenerate. Future research will track the
development of the alimentary canal of adult oestrid flies within the puparium, ideally
combining morphological studies with gene expression analyses. Since the presence of
stem cells in the midgut epithelium of D. melanogaster was revealed, the genetic
control of adult gut development and stem cell maintenance has come to the forefront of
Moreover, given the synapomorphic condition of some organs like the crop or the
cardia for the dipterans (King, 1991; Stoffolano & Haselton, 2013), a better knowledge
of their modifications among oestrid species may contribute positively to studies on
evolutionary and developmental genetics. The underdevelopment of the digestive tract
in adult oestrids may have an evolutionary benefit by putting most available resources
accumulated during the larval stage into sexual maturation, so that adults emerge ready
to reproduce without the need to feed first (Anderson, 2006). Taking into account the short longevity of adult flies in the field (Pitts & Wall, 2004; Anderson, 2006), such life-history strategy may provide adult oestrids with an advantage over other muscoid flies that need a protein intake for sexual maturation after emergence from the puparium. Finally, the surge of interest in the alimentary canal of adult flies in recent years must be emphasised, not only because of the presence of stem cells in the midgut epithelium but also due to its implications for neurobiology, metabolism or immunity studies (Leimatre & Miguel-Aliaga, 2013). A better understanding of the morphology and development of the vestigial alimentary canal of adult oestrids may thus provide valuable new insights into diverse areas of biomedical research.

Acknowledgements
The first author was supported by a fellowship from the University of Alcalá (Ayudas Postdoctorales UAH) and through an award from The Mactaggart Third Fund.

References


**Figure legends**

**Fig. 1.** Micro-CT-based virtual sections of adult *Oestrus ovis* (Oestridae), *Calliphora vicina* (Calliphoridae) and *Hypoderma lineatum* (Oestridae). (A) Anterior cross section of the thorax of a newly emerged *O. ovis* female. (B) Medial cross section of the thorax of a newly emerged *O. ovis* female. (C) Medial cross section of the abdomen of a newly emerged *O. ovis* female. (D) Distal cross section of the abdomen of a newly emerged *O. ovis* female. (E) Distal cross section of the abdomen of a newly emerged *O. ovis* female. (F) Anterior cross section of the thorax of a newly emerged *C. vicina* female. (G) Medial cross section of the thorax of a newly emerged *C. vicina* female. (H) Medial cross section of the abdomen of a newly emerged *C. vicina* female after being fed with pig blood, showing the crop (cr) expanded. (I) Medial cross section of the abdomen of a *C. vicina* female with mature ovaries (ov). (J) Distal cross section of the abdomen of a newly emerged *C. vicina* female. (K) Ventral horizontal section of a newly emerged *H. lineatum* male. (L) Ventral horizontal section of a newly emerged *C. vicina* male after being fed with pig blood, showing the crop (cr) expanded. Abbreviations: (as) air sacs, (ca) cardia, (cr) crop, (dlm) dorsal longitudinal muscles, (dvm) dorsoventral muscles, (fb) fat bodies, (hmg) helicoidal portion of the midgut, (mg) midgut, (sg) salivary glands, (ov) ovaries, (re) rectum, (rp) rectal pouch, (ut) uterus, (va) vagina.

**Fig. 2.** Micro-CT-based 3D-virtual sections of adult *Oestrus ovis* (Oestridae) and *Calliphora vicina* (Calliphoridae). (A) Medial sagittal section of a newly emerged *O. ovis* male. (B) Medial sagittal section of a newly emerged *C. vicina* female after being fed with pig blood, showing the crop (cr) expanded. (C) Ventral horizontal section of a newly emerged *O. ovis* female. (D) Ventral horizontal section of a newly emerged *C. vicina* female. Abbreviations: (as) air sacs, (ca) cardia, (cr) crop, (crd) cropduct, (fb) fat bodies, (hmg) helicoidal portion of the midgut, (mg) midgut, (oe) oesophagus, (rp) rectal pouch, (sg) salivary glands.
Fig. 1. Micro-CT-based virtual sections of adult Oestrus ovis (Oestridae), Calliphora vicina (Calliphoridae) and Hypoderma lineatum (Oestridae). (A) Anterior cross section of the thorax of a newly emerged O. ovis female. (B) Medial cross section of the thorax of a newly emerged O. ovis female. (C) Medial cross section of the abdomen of a newly emerged O. ovis female. (D) Distal cross section of the abdomen of a newly emerged O. ovis female. (E) Anterior cross section of the thorax of a newly emerged C. vicina female. (F) Medial cross section of the thorax of a newly emerged C. vicina female. (G) Medial cross section of the abdomen of a newly emerged C. vicina female after being fed with pig blood, showing the crop (cr) expanded. (H) Medial cross section of the abdomen of a newly emerged C. vicina female after being fed with pig blood, showing the crop (cr) expanded. (I) Medial cross section of the abdomen of a newly emerged C. vicina female with mature ovaries (ov). (J) Distal cross section of the abdomen of a newly emerged C. vicina female. (K) Ventral horizontal section of a newly emerged H. lineatum male. (L) Ventral horizontal section of a newly emerged C. vicina male after being fed with pig blood, showing the crop (cr) expanded. Abbreviations: (as) air sacs, (ca) cardia, (cr) crop, (dlm) dorsal longitudinal muscles, (dvm) dorsoventral muscles, (fb) fat bodies, (hmg) helicoidal portion of the midgut, (mg) midgut, (ov) ovaries, (sg) salivary glands, (ov) ovaries, (re) rectum, (rp) rectal pouch, (ut) uterus, (va) vagina.
Fig. 2. Micro-CT-based 3D-virtual sections of adult Oestrus ovis (Oestridae) and Calliphora vicina (Calliphoridae). (A) Medial sagittal section of a newly emerged O. ovis male. (B) Medial sagittal section of a newly emerged C. vicina female after being fed with pig blood, showing the crop (cr) expanded. (C) Ventral horizontal section of a newly emerged O. ovis female. (D) Ventral horizontal section of a newly emerged C. vicina female. Abbreviations: (as) air sacs, (ca) cardia, (cr) crop, (crd) crop duct, (fb) fat bodies, (hmg) helicoidal portion of the midgut, (mg) midgut, (oe) oesophagus, (rp) rectal pouch, (sg) salivary glands.