

# Histochemical localisation of carbonic anhydrase in the digestive tract and salivary glands of the house cricket, *Acheta domesticus*

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Received: 9 July 2019 / Accepted: 6 October 2019

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RESEARCH ARTICLE

## Abstract

The house cricket (*Acheta domesticus*) is one of several cricket species with great potential to be farmed as a sustainable protein source. In order to succeed in large-scale cricket farming, knowledge of cricket digestion is essential. The digestive tract morphology of *A. domesticus* is well documented, but knowledge of the salivary glands is lacking. In the digestive tract of insects, the carbonic anhydrase (CA) enzyme family is believed to contribute to the luminal pH gradient. Presence of CA in the digestive tract of *A. domesticus* has been reported, but not the cellular localisation. This study examined the digestive tract of *A. domesticus*, including salivary glands, and the cellular localisation and activity of CA in fed or starved (48 h) males and females. Tissues were collected from third-generation offspring of wild *A. domesticus* captured in Sweden and the histology of the salivary glands and the cellular localisation of CA in the digestive tract of *A. domesticus* were determined, to our knowledge for the first time. The salivary glands resembled those of grasshoppers and locusts, and we suggest the two main cell types present to be parietal and zymogenic cells. Histochemical analysis revealed that CA activity was localised in midgut epithelium, both main cell types of salivary gland, and muscle along the entire digestive tract. These findings support the suggestion that CA contributes to digestive tract luminal pH gradient, by driving acidic secretions from the salivary glands and alkaline secretions from the midgut. Starvation resulted in significantly reduced body size and weight, but neither starvation nor sex had any effect on CA activity or localisation.

**Keywords:** histology, gastrointestinal tract, pH, enzyme histochemistry

## 1. Introduction

Crickets (Gryllidae) are easy to breed and can grow well on a variety of feeds, including forage diets and industry by-products (Miech *et al.*, 2016). Species with great potential to be farmed as a sustainable protein source include *Teleogryllus testaceus*, *Gryllus bimaculatus* and *Acheta domesticus* (Miech *et al.*, 2016; Van Huis *et al.*, 2013). Efficiency in animal feeding is based on detailed understanding of the species, its requirements and its ability to utilise feed sources. Thus good knowledge of the anatomy, histology and physiology of the digestive tract is essential in meeting the nutritional requirements of crickets and establishing successful cricket farming.

The general morphology of the digestive tract of *A. domesticus* is well known and has been described in a number of studies (e.g. Elzinga, 1996; Hazelton *et al.*, 1988; Kirby *et al.*, 1982; Kormann and Rutschke, 1976; Srivastava, 1997; Ulrich *et al.*, 1981). However, to our knowledge, the histology of the salivary glands of *A. domesticus* has not been investigated previously. The fine structures of the salivary glands in American grasshoppers (*Rhombalea microptera*, *Melanoplus differentialis*, *Melanoplus femurrubrum*) have been meticulously described by Beams and King (1932) and those in the desert locust (*Schistocerca gregaria*) by Kendall (1969).

The digestive enzymes of *A. domesticus*, in particular amylase, sucrose, maltase, trehalase, lipase and protease,

have been well documented by Teo and Woodring (1994). Additional enzyme families of interest include carbonic anhydrase (CA), which catalyses the reversible reaction  $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+$ . In mammals, CA is found along the entire digestive tract, including salivary glands, and is involved in gastric, pancreatic, biliary and intestinal function (reviewed in Swenson, 1991). There are differences between the sexes with regard to CA activity in certain organs such as the liver (Shields *et al.*, 1983). In crickets, CA has only been detected once, in *A. domesticus*; using electrometric methods, Edwards and Patton (1967) detected CA activity in the foregut, midgut, testes, salivary glands and fat body, and possibly in foregut contents, thoracic muscles and hindgut. They found no differences in CA activity between animals starved for one or four days and non-fasted controls (Edwards and Patton, 1967). However, experiments in rats suggest that several CA isozymes are affected by starvation (Dodgson *et al.*, 1993). The cellular localisation of CA in the digestive tract of *A. domesticus* has not been described previously.

In insect digestion, CA is believed to contribute to the luminal pH gradient in several species (Cooper and Vulcano, 1997; Corena *et al.*, 2005; Edwards and Patton, 1967; Moreira *et al.*, 2017). The pH of the insect digestive tract content gradually changes from acidic in the crop to alkaline at the midgut/hindgut junction, a pattern seen in locusts (*S. gregaria*) (Thomson *et al.*, 1988) and crickets (*Teleogryllus commodus*, *A. domesticus*) (Cooper and Vulcano, 1997; Teo and Woodring, 1994). A number of factors contribute to this pH gradient, including presence and type of feed, acidic salivary secretions (Cooper and Vulcano, 1997), alkaline midgut secretions (Corena *et al.*, 2005; Teo and Woodring, 1985) and digestive tract peristalsis (Teo and Woodring, 1985).

The aim of this study was to describe the digestive tract of *A. domesticus*, including the salivary glands, and determine the cellular localisation and activity of CA in fed or starved (48 h) males and females. The experiment was part of a larger project on farming house crickets in Sweden, using offspring of wild-caught *A. domesticus*.

## 2. Materials and methods

Experiments were performed using young *A. domesticus* adults (1–5 d after final moult) known to be free of *A. domesticus* densovirus (Semberg *et al.*, 2019). The crickets were third-generation offspring of wild individuals collected near Uppsala, Sweden, in September 2017. They were kept in transparent plastic boxes, with mesh on one side for ventilation and enriched with black plastic tubes for shelter, in an environment of 30 °C, 40–50% humidity and 12 h light/dark cycle. The boxes were equipped with cotton-plugged clear plastic vials filled with water and feed was provided *ad libitum*. The feed was a pelleted (cold-pressed) feed

mixture with a crude protein concentration of 192 g/kg dry matter, prepared from commercial wheat flour (30.8%), oat bran (29.6%), wheat bran (22.4%), rapeseed meal (Expro 00SE, 15.0%),  $\text{CaCO}_3$  (1.8%) and vitamin-mineral premix (0.04%). The crickets were divided into two groups of mixed sex: a 'starved' group (12 males and 7 females) received no food, while a 'fed' group (10 males and 10 females) received food *ad libitum*. After 48 h, the crickets were immobilised with  $\text{CO}_2$  and euthanised by decapitation. Crickets were then dissected in cricket Ringer's solution (Woodring and Blakeney, 1980) under a stereomicroscope.

The digestive tract was carefully removed, measured with digital callipers and weighed using an Ohaus Scout Pro balance (Vetek Weighing AB, Vaddö, Sweden). For each individual, sex (male/female), treatment (fed/starved), body weight, digestive tract weight, body length, digestive tract length, crop length, crop width, proventricular length and caecal length were recorded. The digestive tract was divided into one anterior (oesophagus-proventriculus) and one posterior (caecum-rectum) segment. The two salivary glands were removed.

The digestive tract segments and salivary glands were fixed in 2.5% glutaraldehyde in 0.67 mM phosphate buffer, pH 7.2, for 16 h at 4 °C. After rinsing in 0.67 mM phosphate buffer, the samples were dehydrated in graded ethanol at room temperature, embedded in Leica Historesin (Heidelberg, Germany) and serially sectioned at 2 µm thickness using a microtome with a glass blade (Leica RM 2165; Leica Instruments, Wetzlar, Germany). Sections from each sample were stained with haematoxylin and eosin (HE) for histological evaluation. Selected sections of midgut tissue were stained with periodic acid-Schiff (PAS) to detect mucosubstances. The sections were dried on slides and fitted with cover slips using Agar 100 resin. CA was visualised using the Ridderstråle method (Ridderstråle, 1991), which involves incubation of sections floating on a medium containing  $\text{NaHCO}_3$ ,  $\text{CoSO}_4$ ,  $\text{H}_2\text{SO}_4$  and  $\text{KH}_2\text{PO}_4$ , whereby carbon dioxide leaves, pH increases and a cobalt-phosphate-carbonate complex is formed at sites with CA activity. This complex is then converted into a black cobalt-sulphide precipitate. CA inhibitor acetazolamide was added during incubation of one section of all tissues to identify areas with non-specific staining. The sections were counterstained with Azure blue to visualise structures that did not show black staining for CA. The morphology of the digestive tract and salivary glands and the localisation of CA were evaluated using light microscopy. Sections incubated for CA were coded before evaluation.

The effect of sex and treatment on cricket body parameters (body length, digestive tract length, etc.) and CA activity was analysed by one-way analysis of variance (ANOVA) using the GLM procedure in SAS (version 9.4, SAS Institute

Inc. Cary, NC, USA). The following statistical model was used to test the effects and interaction:

$$Y_{ij} = \mu + S_i + T_j + ST_{ij} + e_{ij},$$

where  $Y_{ij}$  is a dependent variable and  $\mu$  is the overall mean of observations,  $S_i$  is the effect of sex,  $T_j$  is the effect of treatment,  $ST_{ij}$  is the interaction between sex and treatment and  $e_{ij}$  is the random residual error. Since there was no significant interaction between sex and treatment, the interaction statement was not used in the final model. Effects were considered significant at  $P \leq 0.05$ .

### 3. Results

#### Effects of starvation on cricket body and digestive tract dimensions

Starvation had a significant effect on body size, with fed crickets being significantly ( $P=0.002$ ) longer and heavier than starved crickets (Table 1). Fed individuals also had a longer digestive tract ( $P=0.017$ ) and tended to have a heavier digestive tract ( $P=0.056$ ) than starved individuals. Female crickets were significantly heavier ( $P<0.001$ ) and had a heavier digestive tract ( $P=0.004$ ) than male crickets. Females also had a longer proventriculus ( $P=0.012$ ) and caecum ( $P=0.011$ ) than male crickets. There was no interaction between sex and treatment ( $P>0.05$ ).

#### Morphology of the digestive tract and salivary glands

The digestive tract morphology of the experimental crickets was similar to that reported in earlier studies on healthy *A. domesticus* (e.g. Elzinga, 1996; Hazelton *et al.*, 1988; Kirby *et al.*, 1982; Kormann and Rutschke, 1976; Srivastava, 1997; Ulrich *et al.*, 1981). The following regions of the digestive tract were included: foregut (oesophagus, crop, proventriculus), midgut (bilateral caeca, ventriculus) and hindgut (ileum, colon, rectum) (Figure 1, Figure 2A-F).

The digestive tract wall consisted of a simple epithelium with underlying basement membrane underlain by striated muscle layers of varying thickness.

The foregut epithelium ranged from flattened in the oesophagus to cuboidal in the proventriculus. Foregut epithelial cells were associated with chitin teeth and/or bristles, predominantly pointing towards the posterior end of the digestive tract (Figure 2A-B). The proventriculus consisted of six longitudinal ridges, with a muscular core, separated by longitudinal chitinous spines and underlain by a muscle layer consisting of two thin longitudinal layers separated by a thick circular layer, approximately 15 fibres in thickness. The midgut epithelium was columnar with no

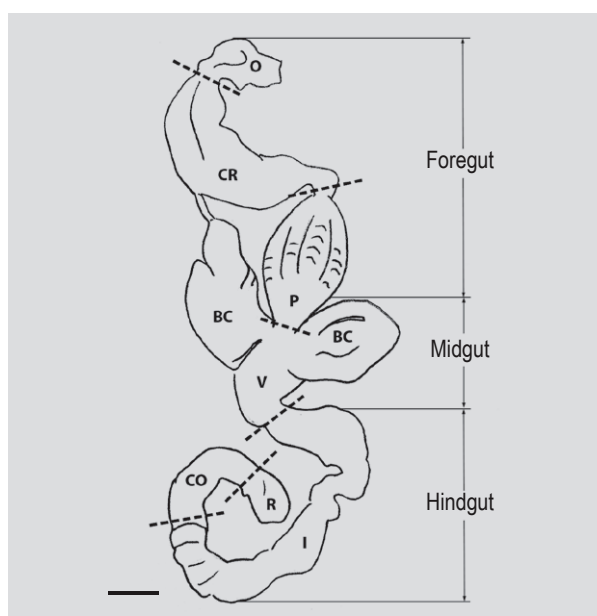
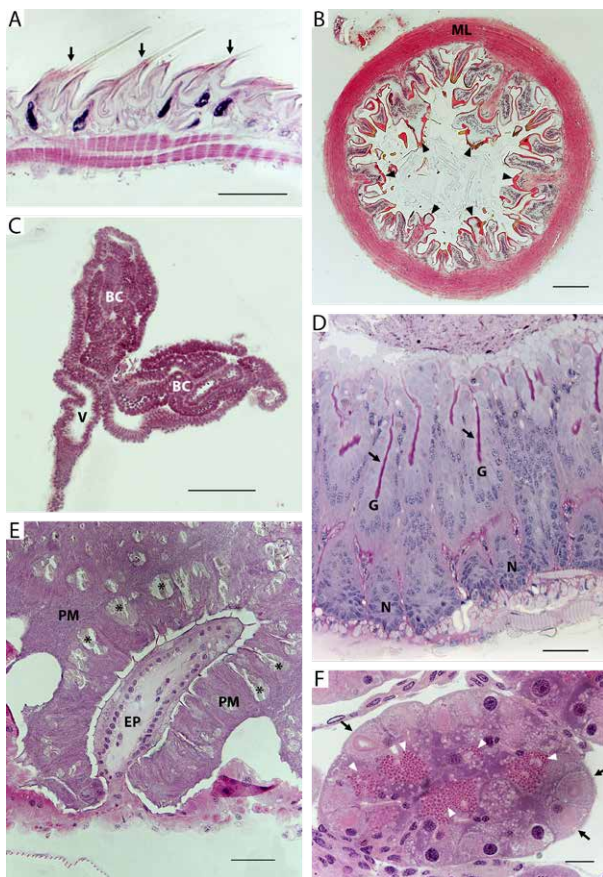


Figure 1. Schematic drawing of the digestive tract of *Acheta domesticus* (bar = 1 mm). Foregut: oesophagus (O), crop (CR), proventriculus (P); midgut: bilateral caeca (BC), ventriculus (V); hindgut: ileum (I), colon (CO), rectum (R).

Table 1. Body and digestive tract dimensions of the house cricket, *Acheta domesticus*. Mean values with standard error ( $\pm$ SE) for female, male, fed and starved young adult crickets (1-5 d after last moult). Significant differences ( $P \leq 0.05$ ) are marked with \*.

	Sex		Treatment	
	F (n=17)	M (n=22)	Fed (n=20)	Starved (n=19)
Cricket length (mm)	19.61 $\pm$ 0.33	18.83 $\pm$ 0.29	20.02 $\pm$ 0.31*	18.43 $\pm$ 0.32*
Digestive tract length (mm)	23.50 $\pm$ 0.76	21.71 $\pm$ 0.66	23.82 $\pm$ 0.68*	21.39 $\pm$ 0.71*
Crop length (mm)	4.782 $\pm$ 0.235	4.440 $\pm$ 0.206	4.658 $\pm$ 0.215	4.565 $\pm$ 0.225
Crop width (mm)	2.666 $\pm$ 0.116	2.468 $\pm$ 0.101	2.605 $\pm$ 0.106	2.529 $\pm$ 0.111
Proventriculus length (mm)	2.628 $\pm$ 0.090*	2.312 $\pm$ 0.078*	2.454 $\pm$ 0.082	2.454 $\pm$ 0.085
Caecum length (mm)	3.560 $\pm$ 0.109*	3.171 $\pm$ 0.095*	3.437 $\pm$ 0.100	3.294 $\pm$ 0.104
Cricket weight (g)	0.431 $\pm$ 0.020*	0.330 $\pm$ 0.018*	0.425 $\pm$ 0.019*	0.335 $\pm$ 0.020*
Digestive tract weight (g)	0.059 $\pm$ 0.005*	0.039 $\pm$ 0.004*	0.055 $\pm$ 0.004	0.043 $\pm$ 0.005



**Figure 2. Digestive tract and salivary glands of *Acheta domesticus*.** (A) Oesophageal epithelium, haematoxylin-eosin (HE) stain (bar = 20  $\mu$ m). Each epithelial cell is associated with a chitin tooth with bristles pointing towards the posterior end of the gut (arrows). (B) Proventriculus, HE, transversal cut (bar = 200  $\mu$ m). The organ consists of six longitudinal ridges (arrowheads) separated by stiff longitudinal chitinous spines and is surrounded by a thick outer circular muscle layer (ML). (C) Midgut, HE, longitudinal cut (bar = 1000  $\mu$ m). Bilateral caeca (BC) and ventriculus (V). Excessive folds of simple columnar epithelium and thin surrounding muscle layer. (D) Midgut epithelium, periodic acid-Schiff stain (bar = 20  $\mu$ m). Gastric glands (G) of midgut epithelium: goblet cells forming crypts containing mucopolysaccharides (arrows). Stem cells assembled in nidi (N). (E) Ileum, HE (bar = 50  $\mu$ m). Club-like epithelial protrusions (EP) and peritrophic membrane (PM) with associated bacteria (\*). (F) Salivary gland acinus, HE (bar = 50  $\mu$ m). Peripheral cells (arrows), central cells (arrowheads).

cuticular intima (Figure 2C-D). Close to the basal lamina, regenerative stem cells were assembled in nidi. Apically of each nidus, adjacent to the lumen, oval cells (goblet cells) were arranged in groups forming crypts. These cells stained very lightly with HE and appeared to contain unstained droplets. In PAS-stained sections, both the lumen of the crypts and the cytoplasmic droplets of the cells surrounding the crypt stained for mucosubstances (Figure 2D). The

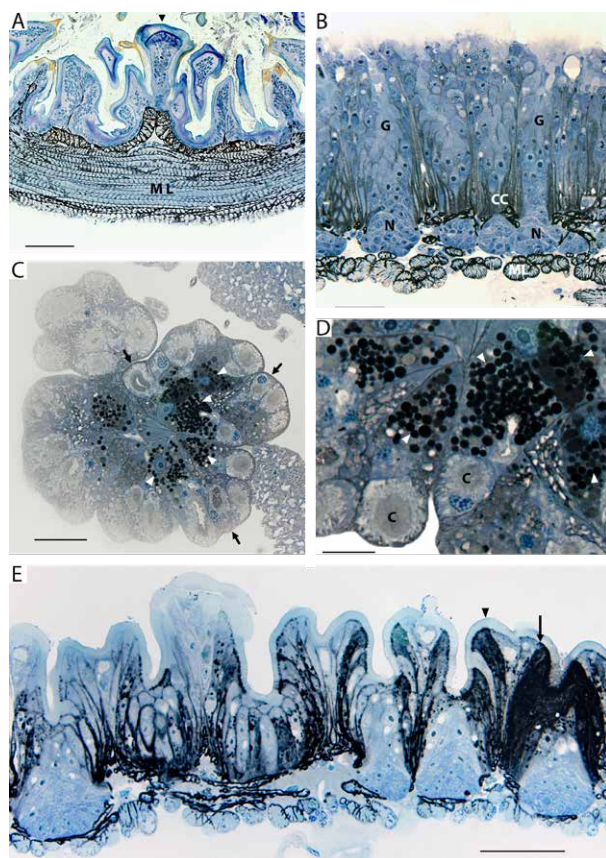
junction between the midgut and the hindgut, referred to as the pyloric valve, was marked. The hindgut epithelium was distinct from the midgut, exhibiting club-like epithelial luminal protrusions with radiating chitinous spines (Figure 2E). The ileum hosted endosymbionts; bacteria associated with the peritrophic membrane and club-like epithelial protrusions. The ileal epithelium, between the protrusions, was simple cuboidal, folded, apically lined with a smooth cuticular intima and underlain by a very thin muscle layer, a few fibres in thickness. The colon and rectum exhibited a simple cuboidal to columnar epithelium apically lined with smooth cuticular intima. The posterior hindgut was underlain by a muscle layer of varying thickness ranging from a few fibres to >10 fibres.

The two salivary glands were located on each side of the anterior oesophagus. They were lobular, finger-like, light yellow to white and could easily be mistaken for fat body. Closer scrutiny revealed that the salivary glands consisted of acini assembled in clusters (Figure 2F). Each acinus had two main cell types, one located in the periphery staining lightly with HE and one located more centrally with marked acidophilic staining. The peripheral cells were round or slightly cone-shaped, with a round peripherally located nucleus. A large round cellular eosinophilic structure with an unstained core was located in the centre of each cell. The inner edges of this structure were poorly defined with a fringed appearance. Adjacent to each peripheral cell was a central cell, triangular in shape, with a most often centrally located round nucleus. The cytoplasm was packed with unstained vesicles and eosinophilic or reddish granules. Occasionally smaller fusiform cells occurred along the outer edges of the acini. These cells had an elongated nucleus and resembled fibroblasts. The salivary ducts were located centrally in each acinus or cluster and consisted of simple cuboidal epithelium with cuticular lining of lamellar appearance. Tracheae were also found in the centre of the clusters or adjacent to the acini.

### Carbonic anhydrase localisation

Control sections incubated with CA inhibitor were generally unstained, but in some sections certain areas contained traces of black stain. These areas contained debris of feed, chitinous structures and bacteria, and were judged not to contain CA.

Digestive tract epithelium: Active CA was detected only in midgut epithelium. In the caeca, black stain was found in the basolateral cell membrane in most columnar cells, fading towards the apical surface of cells (Figure 3B). Throughout the epithelium, there was a repeated pattern of areas with no black staining, which represented nidi and adjacent immature cells. Groups of goblet cells arranged as crypts showed weak membrane-bound basolateral staining. The majority of the ventricular surface epithelium exhibited



**Figure 3. Carbonic anhydrase (CA) localisation in the digestive tract and salivary glands of *Acheta domesticus*.** Active CA is seen as black staining (cobalt precipitation). Azure blue counterstain. (A) Proventriculus (bar = 100  $\mu$ m). Single ridge (arrowhead) consisting of symmetric folds with bristles and a muscular core. Membrane-bound CA in both longitudinal and circular muscle fibres of muscle layer (ML). (B) Midgut epithelium (bar = 50  $\mu$ m). Basolateral membrane-bound CA in columnar (CC) and goblet cells (G). Nidi (N) generally unstained. Strongly membrane-bound CA in muscle layer (ML). (C) Salivary gland acinus (bar = 50  $\mu$ m). Peripheral cells (arrows), central cells (arrowheads). (D) Salivary gland (bar = 50  $\mu$ m). In the peripheral cells, CA is present in a central cellular structure, presumably canaliculi (C). In the central cell (arrowheads), CA is present in granules of the cytosol. (E) Ventricular epithelium (bar = 50  $\mu$ m). Strong basolateral membrane-bound staining for CA in most cells and in cytosolic granules. At the pyloric valve (arrow) membrane-bound staining is seen in the entire membrane, dense black granules fill the entire cell. Narrow apical unstained margin (arrowhead).

the same pattern as in the caeca (Figure 3E). The columnar cells adjacent to the pyloric valve exhibited strong black staining both in the entire membrane and in intracellular dense black granules. In the ventriculus, an unstained, non-descript layer was observed apical to the epithelium.

Salivary glands: CA activity was found in both main cell types of the salivary glands (Figure 3C-D). In the peripheral

cells, weak to moderate black staining was found in the entire membrane and in the periphery of the central cellular structure, leaving the core unstained. In the central cells, CA activity was seen as moderate to strong black staining in the entire membrane and as multiple small dense black granules, of varying number, scattered throughout the cytosol. Fusiform cells, salivary ducts and tracheae showed no signs of CA activity.

Muscle: Membrane-bound CA was found in striated muscle surrounding the epithelium of the digestive tract. CA was demonstrated in both longitudinal and circular muscle fibres of the oesophagus, crop, proventriculus, caeca, ventriculus, ileum, colon and rectum (Figure 3A-B, E). In all these regions CA was present in the entire membrane radiating towards the centre of the muscle fibre forming a spoke-like pattern.

CA activity was classified as absent (-), weak (+), moderate (++) or strong (+++) based on staining intensity. CA staining intensity was not significantly different between the sexes and was unaffected by starvation ( $P > 0.05$ ). This applied to all organs and tissues evaluated. There were no significant interactions between sex and treatment.

#### 4. Discussion

To our knowledge, this is the first histochemical study describing the specific cellular localisation of CA in the digestive tract and salivary glands of *A. domesticus*. We detected CA activity in columnar cells and goblet cells of the midgut epithelium, in both main cell types of the salivary glands and in muscle along the digestive tract. Starvation resulted in significantly reduced body size and body weight, but neither starvation nor sex had any effect on CA staining intensity or localisation.

There are many different isoforms of CA, and they may be membrane-bound, cytoplasmic or even secreted. Vertebrates possess only the  $\alpha$ -CA isoform (Chegwidan and Carter, 2000) whereas both  $\alpha$ - and  $\beta$ -isoforms have been detected in insects (Syrjänen *et al.*, 2013, 2014). The method used for CA localisation in the present study (i.e. cobalt phosphate precipitation for all active CA isozymes; Ridderstråle, 1991) does not allow for identification of different CA isozymes. However, in high-resolution images of the sections it was possible to distinguish between membrane-bound, cytosolic and localised granular areas containing CA.

In the midgut epithelium, CA activity was demonstrated in the basolateral membranes of columnar and goblet cells. In the cells of the pyloric valve, both membranes and intracellular granules were intensely stained for CA. CA localisation and activity has been detected previously in the midgut of the face fly (*Musca autumnalis*) (Darlington

*et al.*, 1985), mosquito larvae (e.g. *Culex quinquefasciatus*, *Anopheles quadrimaculatus*) (del Pilar Corena *et al.*, 2004), *A. domesticus* (Edwards and Patton, 1967) and other insects. In mammals, membrane-bound CA activity of duodenal enterocytes (Lönnerholm *et al.*, 1989) is involved in  $\text{HCO}_3^-$  secretion, contributing to alkalinisation of the gut lumen (Knutson *et al.*, 1995). Edwards and Patton (1967) suggest that CA may affect luminal pH of *A. domesticus* in a similar way. Inhibition of midgut CA in mosquito larvae (e.g. *C. quinquefasciatus*, *A. quadrimaculatus*) resulted in decreased luminal pH (del Pilar Corena *et al.*, 2004). Recently Moreira *et al.* (2017) described an alkalinisation mechanism in the posterior midgut of the mealworm (*Tenebrio molitor*), whereby a  $\text{Na}^+$ -driven  $\text{Cl}^-/\text{HCO}_3^-$  antiporter alkalises the midgut lumen by removal of  $\text{H}^+$  and secretion of  $\text{HCO}_3^-$ . They suggest that cytoplasmic CA gives rise to intracellular  $\text{HCO}_3^-$  by the conversion of circulating  $\text{CO}_2$ . In the present study, we demonstrated presence of CA in membrane and cytoplasm of midgut epithelium in *A. domesticus*, supporting the hypothesis that CA may contribute to the alkaline midgut content of *A. domesticus* observed by Teo and Woodring (1985).

In this study, the histology of the salivary glands of *A. domesticus* was described for the first time. The two salivary glands of *A. domesticus* were located bilaterally of the anterior oesophagus and consisted of two main cell types (peripheral and central cells), organised in clusters of acini. According to Beams and King (1932), the salivary gland acini of grasshoppers (*R. microptera*, *M. differentialis*, *M. femur-rubrum*) consist of parietal cells and zymogenic cells. Kendall (1969) describes the salivary acini of locusts (*S. gregaria*) as also consisting of sheet cells, pigment cells, duct cells and tracheoblasts. Histological evaluation revealed that the acini in young adult *A. domesticus* in this study were similar in structure to those of grasshoppers and locusts. We suggest that the peripheral cells are parietal cells and that the central cells are zymogenic cells. Duct cells, sheet cells and tracheoblasts were identified, but we did not identify pigment cells. The parietal cells of *A. domesticus* in this study had a round, hollow central structure with an indistinct apical border. A similar structure has been described by Beams and King (1932), who suggest that it comprises intracellular canaliculi creating a direct communication with the lumen of the gland.

In mammalian salivary glands, CA is found in secretory granules of serous acinar cells and in striated ductal cells. The enzyme is secreted (Parkkila *et al.*, 1990) and serves to buffer saliva via production of  $\text{HCO}_3^-$  (Leung, 1951). CA is present in the salivary glands of several insect species, including flies (*M. autumnalis* and *Musca domestica*) (Darlington *et al.*, 1985), cockroaches (*Periplaneta americana*) (Hillie and Walz, 2008; Just and Walz, 1994) and *A. domesticus* (Edwards and Patton, 1967). CA localisation and activity have been demonstrated in the peripheral and

duct cells of salivary glands in the cockroach (*P. americana*) (Just and Walz, 1994). CA activity, but not localisation, has been detected by electrometric methods in the salivary glands of *A. domesticus* (Edwards and Patton, 1967). In our study, active CA was detected in the central cellular structure of the peripheral cells (parietal cells) and in the cytoplasmic granules of the central cells (zymogenic cells) of the salivary glands of *A. domesticus*. Edwards and Patton (1967) suggested that CA may contribute to the maintenance of pH in saliva. In a later study on pH regulation in the digestive system of the black field cricket (*T. commodus*), Cooper and Vulcano (1997) suggested that acid crop content is a result of acidic salivary secretions. The role of CA in acidic secretion by mammalian gastric parietal cells, where  $\text{CO}_2$  is converted to  $\text{H}^+$  and  $\text{HCO}_3^-$  by cytosolic CA and  $\text{H}^+$  is then secreted to the lumen via intracellular canaliculi and binds to  $\text{Cl}^-$ , is well known (Davenport and Fisher, 1940; Parkkila, 2000). Our finding of CA activity in cricket salivary glands supports the hypothesis that CA contributes to acidic salivary secretions, especially in light of the finding that inhibition of CA in salivary glands of *P. americana* results in decreased ductal acidification (Hillie and Walz, 2008). Darlington *et al.* (1985) stated that CA is secreted to the lumen of the salivary gland in *M. autumnalis*. It remains to be determined whether the intensely stained intracytoplasmic granules of the central cells in our study secrete CA in a similar way to the CA-containing secretory granules of mammalian salivary glands (Parkkila *et al.*, 1990).

In this study, membrane-bound CA was detected in the muscle fibres along the entire length of the digestive tract. The staining revealed a spoke-like pattern, most likely representing T-tubules. Membrane-bound CA in striated muscle has previously been detected in a wide variety of species, including vertebrates (Geers *et al.*, 1992), crustaceans (Saarikoski and Kaila, 1992) and insects (Seron *et al.*, 2004). CA is suggested to provide interstitial buffering when exercise exceeds the aerobic limit (Saarikoski and Kaila, 1992). The enzyme may also enable large fluxes of lactate across the sarcolemma by producing or buffering  $\text{H}^+$  (Wetzel *et al.*, 2001).

Our morphological findings on the digestive tract and salivary glands of *A. domesticus* originating from wild-caught Swedish specimens are in good agreement with previous work within the field (e.g. Elzinga, 1996; Hazelton *et al.*, 1988; Kirby *et al.*, 1982; Kormann and Rutschke, 1976; Srivastava, 1997; Ulrich *et al.*, 1981). In the midgut epithelium, adjacent to the lumen, oval cells containing PAS-positive droplets arranged as crypts were interpreted to be gastric glands formed by goblet cells. Gastric glands of similar appearance have been described in the midgut of desert cricket (*Melanogryllus desertus*) (Çakici and Ergen, 2012). In our study, fed crickets were significantly heavier and longer than starved crickets, suggesting a decrease

in growth rate when crickets were starved for 48 h and indicating that, although the insects had completed all moults, they had not reached full size. Consequently, adequate feed access past last moult is needed in order for crickets to reach full size.

## 5. Conclusions

This study describes, for the first time, the histology of the salivary glands and the cellular localisation of CA in the digestive tract of *A. domesticus*. Overall, the salivary glands of *A. domesticus* appear to resemble those of grasshoppers and locusts. We suggest that the two main salivary gland cell types, peripheral and central, are parietal and zymogenic cells, respectively. The CA enzyme family was found to be present in midgut epithelium, both main cell types of salivary glands and muscle layers along the entire digestive tract. We suggest that CA may contribute to the digestive tract luminal pH gradient by driving acidic secretions from the salivary glands and alkaline secretions from the midgut. Based on our findings, CA activity does not appear to depend on short-term starvation or sex in adult *A. domesticus*. Digestive tract CA may have a fundamental/homeostatic role and is thus unaffected by gut contents and/or changes in metabolic activity.

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