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Research Article

Improved Visualization of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)—Part II: Alimentary Canal Components and Measurements

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Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) is a pest of stored food products and problematic to every type of poultry production facility. Larvae and adults can ingest and harbor foodborne and poultry pathogens. Determining the efficiency of this insect's capacity to transmit disease is critical to improving management of *A. diaperinus* on poultry facilities and providing a safe food supply for human consumption. However, a deficiency exists in the literature reporting measurements of the gut and its defined segments. Previous reports include line drawing depictions, which aid little in the determination of the pathogen reservoir potential of these insects. Advances in technology allowed more accurate visualization and precise measurement of gross anatomical features of the alimentary canal. A photographic depiction to aid the researcher in the visualization of anatomical features and accurate measurements of the alimentary canal for these insects is presented here.

1. Introduction

The of high-density poultry feeding operations to increase production output in order to meet market demand has changed the environment in which poultry is raised. The close quarters and high bird density favors the survival of arthropod pests. *Alphitobius diaperinus* (Panzer) (1797) (Coleoptera: Tenebrionidae), a pest of stored food products, is a common and persistent pest in every type of poultry production facility: breeders, grow-out, caged-layers and pullets. These insects have adapted well to the artificially controlled environment within poultry houses and what were once only minor pests in low density flocks have become large infestations in high-density rearing facilities. Their presence generates economic and management concerns. For example, *A. diaperinus* survive on the floor of a broiler production house in the accumulated mix of bedding material, excreta, feathers, spilt feed, carcasses, and other debris, referred to as litter. The high density of birds in production results in increased litter moisture, both from the excreta and automatic drinkers. Combined with the controlled temperature in the houses these conditions are highly conducive to beetle survival and population expansion.

Chickens and turkeys readily feed on *A. diaperinus*, and young birds preferentially ingest larvae, even in the presence of starter feed [1, 2]. Chicks fed solely *A. diaperinus* larvae for 9 days gained 37% less body weight than chicks on starter feed and, in addition, showed signs of stress [2]. This weight was not recovered when returned to starter feed through 14 days of age [2]. The omnivorous diet of *A. diaperinus* also means that they can compete with the birds for their feed. Furthermore, *A. diaperinus* can ingest and harbor foodborne and poultry pathogens (reviewed in: [3]). Consequently, *A. diaperinus* represents a health issue to the birds and to the humans which consume the birds [4].

During their life cycle *A. diaperinus* larvae migrate into the insulation of the building walls and the soil beneath the litter for pupation and eventual eclosion. Their tunneling

behavior disrupts the compacted earth floors and dense wall insulation [5]. Their activity in the walls reduces the building insulating capacity, causing enough damage to raise energy costs 67% and require replacing of insulation every two to three years [6–9]. Their activity in the compacted earthen floors, on which bedding is spread, results in an irregular and hollowed floor surface [6]. This hollowing can retain bedding and reduce the effectiveness of litter clean outs by tractor loaders. Therefore, *A. diaperinus* also represent a structural pest for the producer. Economic effects of *A. diaperinus* infestations on poultry production are difficult to quantify. Of financial concern for the producer is the issues that these insects cause facilities structural damage, affect bird growth and health, and vector poultry diseases.

A primary concern for the consumer is that these insects vector foodborne pathogens. Determination of the efficiency of this insect's capacity to harbor pathogens is critical to improving management of *A. diaperinus* in poultry facilities and providing a safe food supply for human consumption. Understanding the anatomy and physiology of the insect to model pathogen movement and transport within the insect is vital [10–12]. Line drawings of the alimentary canal for larvae and adult *A. diaperinus* have been provided in previous studies, but these aid little in determining reservoir potential of the alimentary canal [13–15]. No single reference exists with a photographic depiction to aid the researcher in the visualization of anatomical features and measurements of the alimentary canal for these insects. Improved reference images are needed to more accurately describe the larval and adult *A. diaperinus* alimentary canal, and these are presented here.

2. Experimental

2.1. Beetles. The Southern Plains Agricultural Research Center (SPARC) starter colony of *A. diaperinus* was from a colony originally isolated from a poultry farm located in Wake County, NC. The SPARC colony has remained in production since 2004. The adult colony was reared in 1000 mL wheat bran (Morrison Milling Co., Denton, TX) in plastic containers (15 × 15 × 30 cm) with screened bottoms. Insects were provided a 6 cm^2 sponge, placed atop a piece of aluminum foil, and moistened with deionized water as needed, and a 0.5 cm thick slice of a medium-sized apple was replenished twice per week. Fishmeal (30 mL; Omega Protein, Inc., Hammond, LA) was added to the wheat bran once per week, and new wheat bran was added as it was depleted by dropping through the screened bottom of the cage. Eggs were collected as needed on layered black construction paper (6 × 6 cm) and transferred to a separate container; emergent larvae were maintained as described above in a solid bottom container, until pupation. Pupae were transferred to a screened bottom container and emerging adults reared as described above. The entire colony was maintained at 30°C in an 8 : 16 hr (light : dark) photoperiod.

2.2. Morphometrics of Insects and Alimentary Canal

2.2.1. Insect Measurements. Immediately before dissection for removal of alimentary canals, as described below, male and female adults and late instars were measured using imagery software described by Esquivel [16]. Head capsule widths were also recorded for larvae to determine stadia, and, based on previous head capsule width measurements [17], the larvae used in this study were 7th instar or older and the adults were more than 4 weeks after eclosion.

2.2.2. Alimentary Canal Measurements. To determine size and capacity of the sections comprising the alimentary canals, intact alimentary canals were removed from male (n = 5) and female (n = 5) adults and late instars (n = 10). Equipment and dissection methodologies described by Esquivel [16] were slightly modified for excision of alimentary canals. Briefly, individual specimens were examined under an Olympus SZ60 dissecting stereomicroscope (Olympus, Kalamazoo, MI, USA). Lumenera INFINITY software and INFINITY 1–3 C camera (Lumenera, Ottawa, ON, Canada) were interfaced with a computer to record images and measurements of each specimen. Because the adults and larvae *A. diaperinus* were smaller than those insects examined previously, dissection technique, pins, and forceps varied, as described below.

2.2.3. Adults. Beetles were taken from rearing cages and placed in a vial at −20°C for ca. 15 min. Individual adults were removed from the vial and pinned (no. 00, BioQuip, Rancho Dominguez, CA, USA) dorsolaterally through the right elytron and through the body. Positioning of the pinning site was closer to the right margin of the abdomen to prevent piercing of the alimentary canal. Pinning at this location also provided an "anchor" during the dissection process. The beetle was then pinned into one of the "dissection wells" [16]. Distilled (RO) water was added to the well to facilitate dissection and excision of the alimentary canal.

The technique to remove the wings and abdominal dorsal cuticle was similar to Esquivel [16], with the exception that the right pair of wings was not removed and the cuticle was cut only at the left lateral margin of the abdomen. Two pair of forceps (no. 55 Rubis, BioQuip, Rancho Dominguez, CA, USA) were used to grasp the thorax dorsally at the midline and break each half open, allowing access to the ventral connective tissue between the head and the thorax. This connective tissue was severed to allow removal of the head intact and the alimentary canal was excised by teasing away the tracheae and connective tissue along the length of the body. The tissue between the ultimate and penultimate ventral abdominal plates was severed, allowing removal of the intact alimentary canal. The intact canal was then placed into a separate well and the head was grasped dorsally at midline and gently pried open. Pieces of the exoskeleton and tissue were teased away leaving only the mandibles and alimentary canal. Similarly, abdominal plates still attached around the rectum were teased away. The mandibles were pinned using minuten pins (BioQuip, Rancho Dominguez, CA, USA) and the rectum was grasped and pulled taut to lay the alimentary canal in a straight line, exercising care to not distend the canal past its normal length. A minuten pin held the rectum in place.

Following distension of the alimentary canal, measurements were recorded for the foregut (from the mouth—including buccal cavity, pharynx, and esophagus—to distal end of proventricular valve), the midgut (distal end of proventricular valve to distal end of pyloric valve), the small intestine (distal end of pyloric valve to enlargement of the intestine), the large intestine (enlarged intestine), and the rectum. Section assignments closely follow designations of McAllister et al. [13] and Snodgrass [18] except the rectum, which was not delineated or measured separately in those studies.

Total exterior body lengths ($n = 10$ per group) were measured along the anteroposterior axis for comparison to total alimentary canal lengths. For the adult, the head was measured from the anterior end of head to the first anterior thoracic segment. The thorax was measured from the first anterior thoracic segment to the anterior elytra attachment. The abdomen was measured from the anterior elytra attachment to the distal end of abdomen. For the late instar a measurement from the anterior end of head to the distal end of abdomen was performed.

2.2.4. Larvae. Late instars were taken from rearing cages and placed in a modified plastic centrifuge vial (JFE, unpublished data) and killed by exposure to ethyl acetate for 10–15 min. Dead larvae were placed in a dissection well and held down using a modified no. 00 pin (JFE, unpubl. data) allowing anchoring of the larva so that the alimentary canal was not pierced by the conventional pinning technique. Distilled water was added to the well to facilitate dissection and excision of the alimentary canal.

The dorsal cuticle of the larvae was cut along the left margin from the penultimate abdominal segment to the first thoracic segment. The cuticle was pulled to the right while removing tracheae and other tissue. Similar to the adults, the head was removed intact from the larva and the last abdominal segment (i.e., pygidium) was also removed intact. Removal of the alimentary canal from the body, subsequent clearing of the attached material (head and abdominal segments), distension of the alimentary canal, and measurement of alimentary canal sections were as described for adults.

Total exterior body lengths ($n = 10$) were measured along the anteroposterior axis for comparison to total alimentary canal lengths. For the late instar, a measurement from the anterior end of head to the distal end of abdomen was performed.

2.3. Data Analysis. Data were analyzed using commercially available statistical software (Prism ver. 5.01, GraphPad Software Inc., La Jolla, CA). Descriptive statistics were generated and are presented in table formats. Within each anatomical segment, life stage, and sex of insect, a means comparison of length was performed using a two-way ANOVA followed by Bonferroni posttests ($P < 0.05$).

3. Results and Discussion

The digestive tract is arranged into fore-, mid-, hindgut and rectum sections, which can be easily demarcated by visual examination. Figures 1(a) and 1(b) of *in situ* and extracted digestive tracts reveal the simplistic gut structure with a sigmoid bend distinctive to larval *A. diaperinus.* Conversely, adult *A. diaperinus* possess a more convoluted alimentary canal containing a complete loop before reaching the pyloric valve (Figures 1(c) and 1(d)). These images correspond with existing line drawn schematics presented by McAllister et al. [13] and Rahman et al. [14, 15] but provide more details regarding morphology and reservoir potential of the alimentary canal. Figure 2 displays the distended adult alimentary canal used during measurement of gut segments and insets of the proventricular valve and expanded views of the female and male rectum. Figure 3 displays the extracted larval alimentary canal demonstrating features used to delineating gut segments, with insets of the demarcating proventricular and pyloric valves, and the distended position of hindgut showing features used during measurement of the large and small intestinal segments. These delineating features are similar on both the larva and adult.

The alimentary canal of *A. diaperinus* is a tubular structure with similarities to beetles consuming stored grain products, as well as characteristics distinctive to its more omnivorous feeding habits when in a poultry production environment, as previously described by McAllister et al. [13]. Larvae in particular are known to be cannibalistic and have chitinase activity in their digestive secretions [19]. However, a study comparing the alimentary canal of *A. diaperinus* of individuals fed herbivorous or carnivorous diets determined no anatomical differences between the larvae on the two disparate diets [15]. Therefore our laboratory-raised insects were used as representative specimens for this study.

Adults of 2 to 4 months of age and late instar larvae were measured for this study. The mean head capsule width of the late instars ($n = 10$) used in this study was 1.084 ($\pm$ 0.013) mm and ranged from 0.960 to 1.320 mm; representing 7th to 9th instar. Barké and Davis [17] reported head capsule widths of 0.95, 1.08, and 1.28 mm, and Francisco and Prado [20] reported widths of 1.061, 1.208, and 1.339 mm for 7th, 8th, and 9th instars, respectively. Overall measurements of the alimentary canal and its sections resemble those reported by McAllister et al. [13] for fully tanned adults and 8th- to 11th-instar larvae. However, some variation was expected due to the likely differences in the range of ages of the insects used in the two studies.

The ten adult foreguts in this study, measured from the epipharynx to the posterior end of the proventricular valve, averaged 1.73 mm in length and the ten late instars foreguts averaged 2.36 mm (Table 1). No significant differences in the length of the foregut between male and female, nor between adult and late instar, were found. In comparison, foregut measurements from the mouth to the proventricular valve previously yielded mean lengths of 1.25 mm in adults and 1.5 mm in larvae (McAllister et al. [13]). However, measurements by McAllister et al. [13] were made using a dissecting scope and a calibrated ocular micrometer which may have affected precision.

The midgut was demarcated from the distal end of proventricular valve to the distal end of pyloric valve and measured 9.98 mm in larvae and 9.00 mm adults after distension.

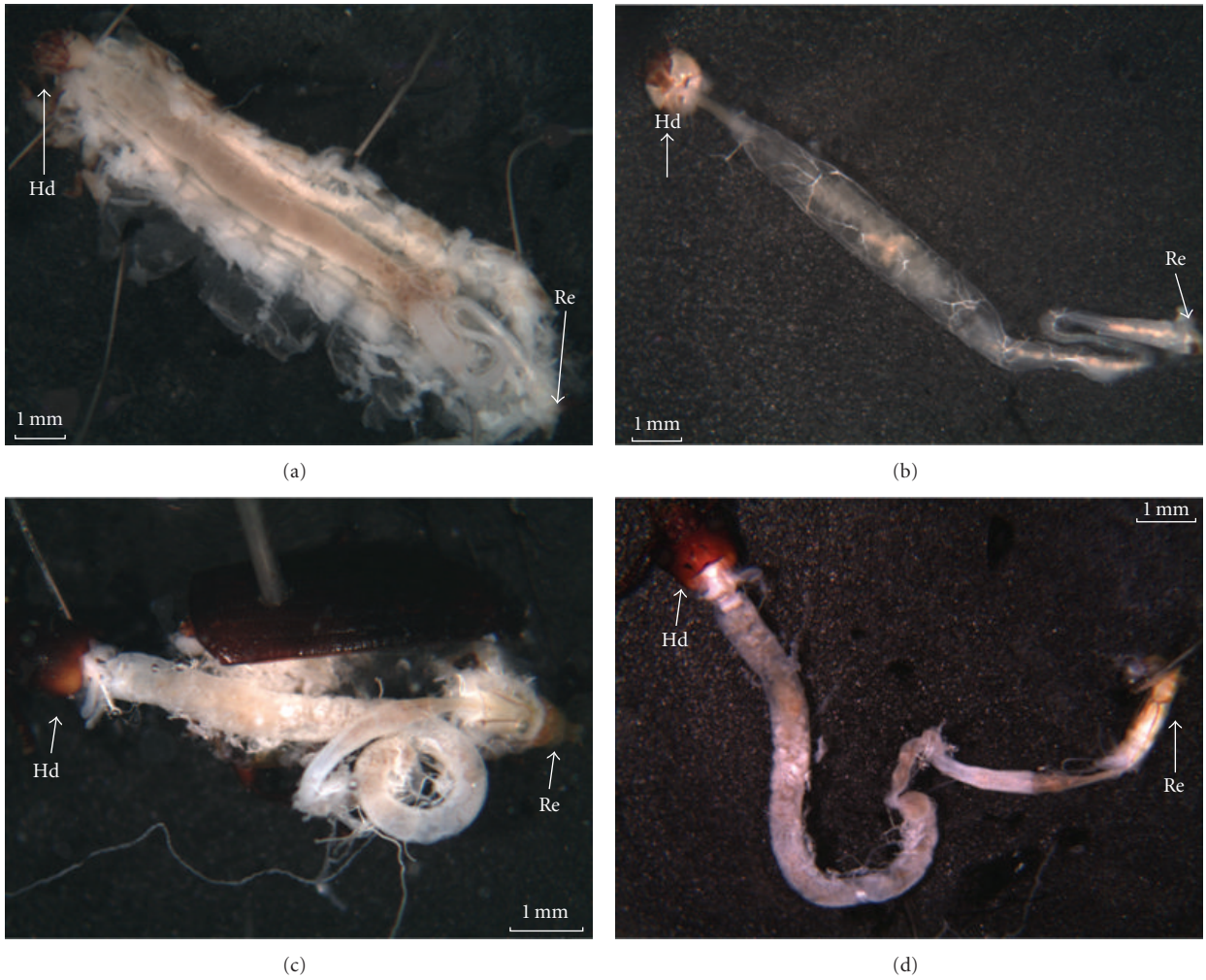

Figure 1: Alimentary canals for larval and adult *Alphitobius diaperinus*: ((a) and (b)), larval canal *in situ* and extracted, respectively; ((c) and (d)) adult canal *in situ* and extracted, respectively. Hd: head; Re: rectum.

Table 1: Mean length (mm) and standard deviation (SD) of alimentary canal of the *Alphitobius diaperinus* female and male beetles (>4 weeks post eclosion) and the late instars (7–9th). Measured by microscopy.

	Foregut		Midgut		Small intestine		Large intestine		Rectum		Total alimentary canal	
	Length	SD	Length	SD	Length	SD	Length	SD	Length	SD	Length	SD
Adult female	1.70	$\pm 0.30^{a}$	9.22	$\pm 0.71^{a,b}$	3.24	$\pm 0.35^{a}$	2.30	$\pm 0.18^{a}$	2.29	$\pm 0.32^{a}$	18.74	$\pm 1.11^{a}$
Adult male	1.75	$\pm 0.12^{a}$	8.77	$\pm 1.89^{a}$	3.14	$\pm 0.40^{a}$	2.43	$\pm 0.32^{a}$	1.26	$\pm 0.19^{a,b}$	17.35	$\pm 1.83^{a}$
Late instar	2.36	$\pm 0.38^{a}$	9.98	$\pm 1.94^{b}$	3.50	$\pm 0.46^{a}$	2.14	$\pm 0.50^{a}$	0.64	$\pm 0.10^{b}$	18.61	$\pm 2.30^{a}$
Adult*	1.73	±0.22	9.00	±1.37	3.19	±0.36	2.36	±0.25	1.78	±597.5	18.04	±1.61

* Mean compilation of adult male and adult female measurements.
[a-b]Sample groups (late instar, female, and male) with the same letter are not significantly different ($P < 0.05$) as compared within the anatomical segment of the gut (nonparametric 2-way ANOVA with Bonferroni posttests).

A significant difference was found in the length of the midgut between the male, which was shorter than that of the late instar. In comparison, McAllister et al. [13] defined the midgut as extending from the proventricular valve and terminating at the pyloric valve, measuring 7.5 mm in larvae and 4.1 mm adults.

The hindgut is divided into a small and large intestine. The small intestine was demarcated from the distal end of pyloric valve extending to the enlargement of the canal, signifying the start of large intestine, and measured 3.50 mm in larvae and 3.19 mm adults. The large intestine was demarcated from the enlargement of intestine to the origination of

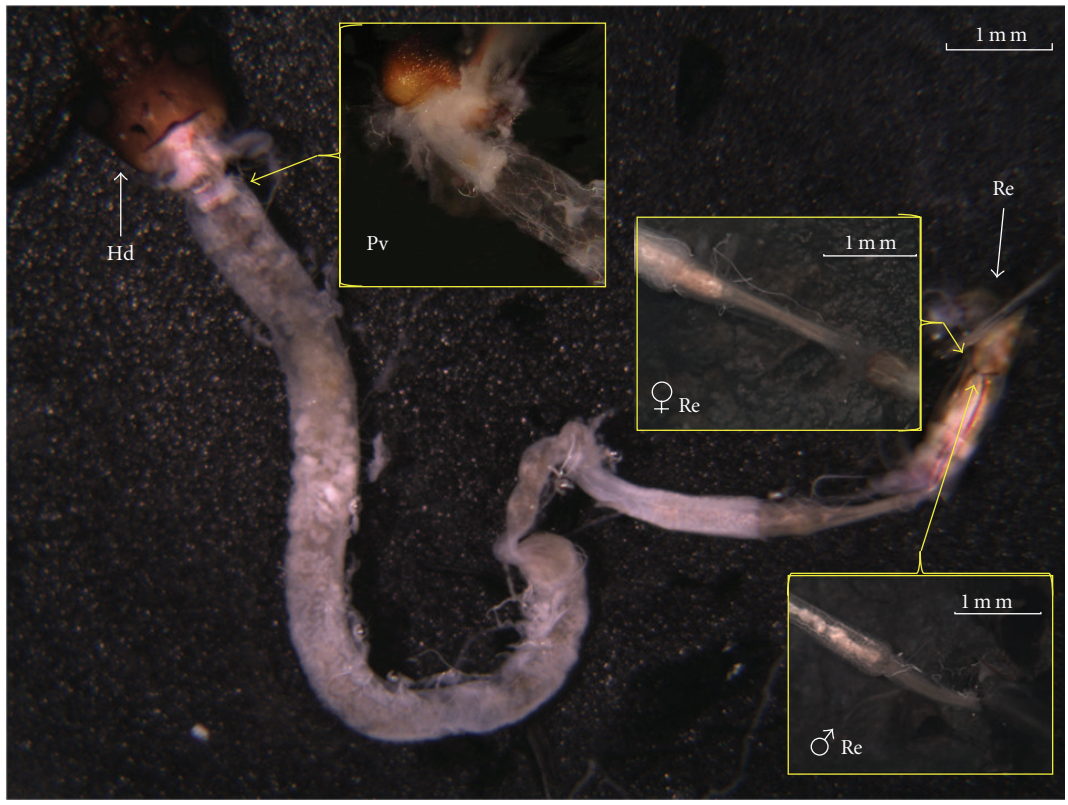

Figure 2: Distended alimentary canal of adult *Alphitobius diaperinus* canal; yellow lines indicate area of expanded detail for Pv: proventricular valve, male and female rectal areas. Hd: head; Re: rectum.

the rectum and measured 2.14 mm in larvae and 2.36 mm adults. No significant differences were found in the length of the hindgut between male and female, nor between the adult and late instar. In comparison, McAllister et al. [13] defined the anatomy in the larvae of the small intestine as a straight tube which begins near the pyloric valve and extends anteriorly to the posterior margin of the fifth abdominal segment for a total length of 2.0 mm. At that point, it reverses direction and extends posteriorly as the large intestine for a length of 2.9 mm. In the adult, the small intestine was described as a single loop beginning from the pyloric valve and extending to the posterior margin of the third abdominal segment for a length of 3.5 mm. It then extends posteriorly as the large intestine for a length of 2.4 mm.

The rectum was also measured as demarcated from the posterior end of large intestine to the anus and measured 0.64 mm in larvae and 1.78 mm adults. The female rectum was significantly longer than that of the late instar. Neither the ovipositor of the female nor the aedeagus of the male was included in these measurements; their anatomy is discussed in a counterpart paper in this journal issue [21].

The largest observed discrepancy was in the length of the midgut. McAllister et al. [13] reported lengths of 7.5 and 4.1 mm for larvae and adults, respectively, while current results indicated lengths of 9.98 and 9.00 mm for larvae and adults, respectively. Methodology differences between studies may account for these differences. In the current study, the alimentary canal was distended to normal length, to ensure a straight line measurement. However, methodologies in McAllister et al. [13] did not clearly indicate measurement technique and suggests measurements of the alimentary canal as it lay *in situ*. Discrepancies in measurements may also be attributed to definitions of sections comprising the alimentary canal. In the current study, measurements involving the proventricular and pyloric valves reached to the distal side of the valve. In contrast, language in McAllister et al. [13] suggests that measurements were taken from the proximal side of the respective valves. Inclusion of the valves in the measurements would likely bring their estimates closer to those reported in the current study.

According to Dunford and Kaufman [22] the average length of an adult *A. diaperinus* is approximately 5.8 to 6.3 mm, therefore the fore-, mid-, and hindguts are more than 2.5 times the length of the insect. Barké and Davis [17] noted that average adult female ranged from 6.75 to 8.00 mm and male from 5.50 to 7.00 mm; however, the method of measurement collection was not presented. Rahman et al. [14] used a micrometer to measure characteristics of the adult and determined the foregut was "about" 2 mm in length and the hindgut (including the rectum) was 0.9 cm. The entire canal was reported to be 3 times the body length, 21 mm in the female (body length of 7 mm) and 19 mm in the male (body length of 5 mm). The average length of an adult beetle, in this study, was 7.01 mm, and the average

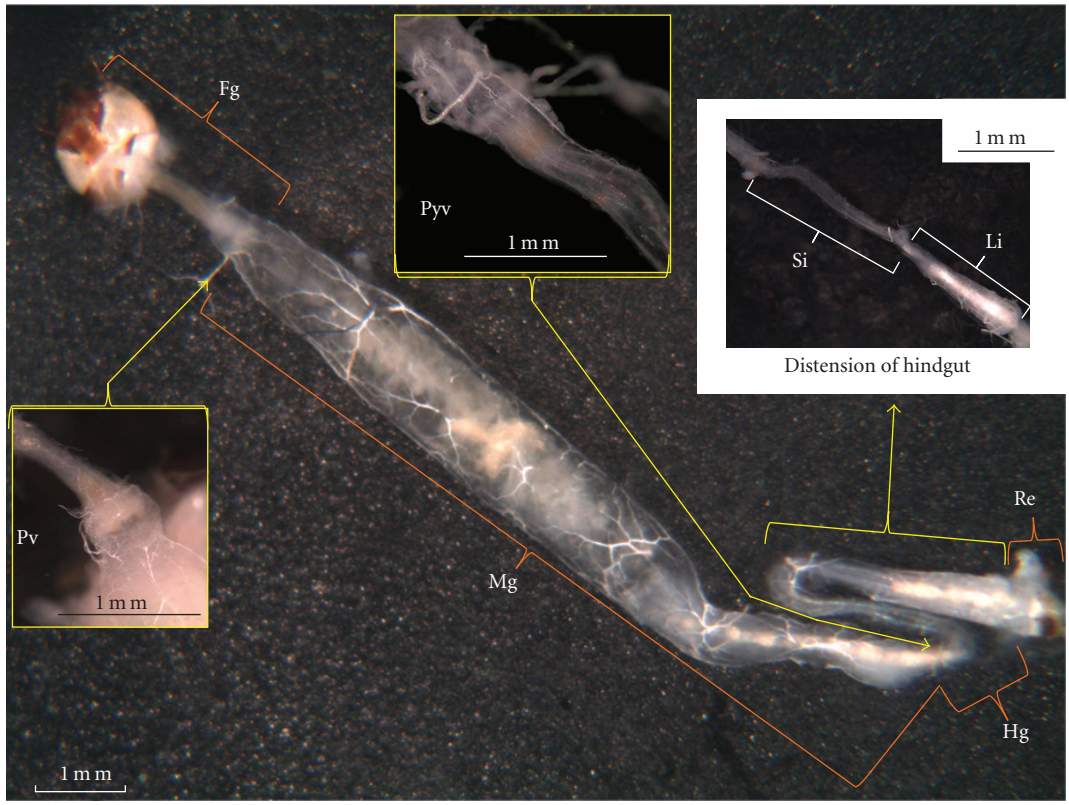

Figure 3: Components of larval *Alphitobius diaperinus* alimentary canal; yellow lines indicate area of expanded detail. Fg: foregut; Hg: hindgut; Li: large intestine; Mg: midgut; Pv: proventricular valve; Pyv: pyloric valve; Re: rectum; Si: small intestine.

Table 2: Mean length (mm) and standard deviation (SD) of segment and total body length of *Alphitobius diaperinus* beetles and total body length of late instars (7–9th). Measured by microscopy on the anteroposterior axis.

Segment	Female ($n = 10$)		Male ($n = 10$)		Late instars ($n = 10$)	
	Length	±SD	Length	±SD	Length	±SD
Head	1.094	±0.008[a]	1.165	±0.013[a]	—	
Thorax	1.355	±0.010[a]	1.382	±0.008[a]	—	
Abdomen	4.682	±0.030[b]	4.333	±0.020[b]	—	
Total	7.131	±0.041[c]	6.883	±0.031[c]	12.80	±1.169[d]

[a–d]Sample groups (late instar, female, and male) with the same letter are not significantly different ($P < 0.05$) (nonparametric 2-way ANOVA with Bonferroni posttests).

adult female ranged from 6.49 to 7.77 mm and male from 6.50 to 7.42 mm (Table 2). The mean alimentary canal length (foregut through rectum) was 2.6 times the length of the adult insect (Table 3).

The mean length of the late instars was 12.80 mm, ranging from 9.81 to 14.78 mm (Table 2), and the total alimentary length was 1.5 times the body length of the insect (Table 3). According to Dunford and Kaufman [22], the average length of a late instar was approximately 7 to 11 mm in length and the fore-, mid-, and hindguts were 1.6 to 2.5 times the length of the insect. Rahman et al. [15] determined larval alimentary canal length 1.5 times (21 mm) that of an 8th-instar body length (14 mm). They also stated that fore-, mid-, and hindgut measurements were 2, 12, and 7 mm, respectively; however the method of measurement collection was not presented. In addition, rectal lengths were included in the hindgut measurement.

4. Conclusions

A handful of studies have reported measurements of various parts of the alimentary canal of *A. diaperinus*. However, the exact method used for measurement and the anatomical structures used to define segment features were not always provided. No single study encompassed the scope of measurements on the same group of insects presented in this study. Advances in current technology also allowed more accurate visualization and precise measurement of gross anatomical features of the alimentary canal. These images and measurements provide additional perspective on the

Table 3: The length of the segments of the alimentary canal as percent of total alimentary canal length (ACL) and body length (BL) of the *Alphitobius diaperinus* female and male beetles (>4 weeks after eclosion) and the late instars (7–9th). Measured by microscopy.

	Foregut		Midgut		Small intestine		Large intestine		Rectum		Total alimentary canal
	ACL	BL	ACL	BL	ACL	BL	ACL	BL	ACL	BL	BL
Adult female	9.0	23.8	49.2	129.1	17.3	17.3	12.2	32.1	12.2	32.1	262.5
Adult male	10.1	25.5	50.6	127.4	18.1	18.1	14.0	35.2	7.3	18.3	252.0
Late instar	12.7	18.4	53.6	77.9	18.8	27.4	11.5	16.7	3.4	5.0	145.4
Adult*	9.6	24.6	49.9	128.3	17.7	45.5	13.1	33.6	9.9	25.4	257.1

* Compilation of adult male and adult female measurements.

pathogen reservoir potential of *A. diaperinus* and the magnitude of potential disease agents which could be harbored.

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References

[1] J. L. Despins and R. C. Axtell, "Feeding behavior and growth of turkey poults fed larvae of the darkling beetle, *Alphitobius diaperinus*," *Poultry Science*, vol. 73, no. 10, pp. 1526–1533, 1994.

[2] J. L. Despins and R. C. Axtell, "Feeding behavior and growth of broiler chicks fed larvae of the darkling beetle, *Alphitobius diaperinus*," *Poultry Science*, vol. 74, no. 2, pp. 331–336, 1995.

[3] T. L. Crippen and T. L. Poole, "Lesser mealworm on poultry farms: a potential arena for the dissemination of pathogens and antimicrobial resistance," in *On-Farm Strategies to Control Foodborne Pathogens*, T. R. Callaway and T. S. Edrington, Eds., Nova Science, New York, NY, USA, 2012.

[4] A. J. Roche, N. A. Cox, L. J. Richardson et al., "Transmission of Salmonella to broilers by contaminated larval and adult lesser mealworms, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)," *Poultry Science*, vol. 88, no. 1, pp. 44–48, 2009.

[5] K. C. Stafford, C. H. Collison, J. G. Burg, and J. A. Cloud, "Distribution and monitoring lesser mealworms, hide beetles, and other fauna in high-rise, caged-layer poultry houses," *Journal of Agricultural Entomology*, vol. 5, no. 2, pp. 89–101, 1988.

[6] C. J. Geden and R. C. Axtell, "Factors affecting climbing and tunneling behavior of the lesser mealworm (Coleoptera: Tenebrionidae)," *Journal of Economic Entomology*, vol. 80, no. 6, pp. 1197–1204, 1987.

[7] C. J. Geden and J. A. Hogsette, "Research and extension needs for integrated pest management for arthropods of veterinary importance," in *Proceedings of a Workshop in Lincoln, Nebraska*, pp. 1–328, April 1994.

[8] R. D. Safrit and R. C. Axtell, "Evaluations of sampling methods for darkling beetles (*Alphitobius diaperinus*) in the litter of turkey and broiler houses," *Poultry Science*, vol. 63, no. 12, pp. 2368–2375, 1984.

[9] J. A. Vaughan, E. C. Turner, and P. L. Ruszler, "Infestation and damage of poultry house insulation by lesser mealworms, *Alphitobius diaperinus* (Panzer)," *Poultry Science*, vol. 63, pp. 1094–1100, 1984.

[10] T. L. Crippen and T. L. Poole, "Conjugative transfer of plasmid-located antibiotic resistance genes within the gastrointestinal tract of lesser mealworm larvae, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)," *Foodborne Pathogens and Disease*, vol. 6, no. 7, pp. 907–915, 2009.

[11] T. L. Crippen, C. L. Sheffield, S. V. Esquivel, R. E. Droleskey, and J. F. Esquivel, "The acquisition and internalization of *Salmonella* by the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)," *Vector-Borne and Zoonotic Diseases*, vol. 9, no. 1, pp. 65–71, 2009.

[12] T. Poole and T. Crippen, "Conjugative plasmid transfer between *Salmonella enterica* Newport and *Escherichia coli* within the gastrointestinal tract of the lesser mealworm beetle, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)," *Poultry Science*, vol. 88, no. 8, pp. 1553–1558, 2009.

[13] J. C. McAllister, C. D. Steelman, and C. E. Carlton, "Histomorphology of the larval and adult digestive systems of *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)," *Journal of the Kansas Entomological Society*, vol. 68, pp. 195–205, 1995.

[14] F. Rahman, S. Hussain, and R. Rahman, "Alimentary canal and malpighian tubules of the adult *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)," *Bangladesh Journal of Zoology*, vol. 19, no. 1, pp. 21–28, 1991.

[15] F. Rahman, S. Hussain, and R. Rezaur, "The larval alimentary canal and malpighian tubules of *Alphitobius diaperinus* Panzer (Coleoptera:Tenebrionidae)," *Bangladesh Journal of Zoology*, vol. 18, no. 2, pp. 215–222, 1990.

[16] J. F. Esquivel, "Improved visualization of fat body cell conditions and abundance in the southern green stink bug (Hemiptera: Pentatomidae)," *Journal of Entomological Science*, vol. 46, no. 1, pp. 52–61, 2011.

[17] H. E. Barké and R. Davis, "Notes on the biology of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)," *Journal of the Georgia Entomological Society*, vol. 4, no. 2, pp. 46–50, 1969.

[18] R. E. Snodgrass, "The organs of ingestion," in *Principles of Insect Morphology*, Cornell University Press, Ithaca, NY, USA, 1993.

[19] S. C. Saxena and K. Sarin, "Chitinase in the alimentary tract of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)," *Applied Entomology and Zoology*, vol. 7, no. 2, article 94, 1972.

[20] O. Francisco and A. P. do Prado, "Characterization of the larval stages of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) using head capsule width," *Brazilian Journal of Biology*, vol. 61, no. 1, pp. 125–131, 2001.

[21] J. F. Esquivel, T. L. Crippen, and L. A. Ward, "Improved visualization of *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae): I. Morphological features for sex determination of multiple stadia," *Psyche*. In press.
[22] J. C. Dunford and P. E. Kaufman, "Lesser mealworm, litter beetle *Alphitobius diaperinus* (Panzer) (Insecta: Coleoptera: Tenebrionidae)," in *Featured Creatures*, T. R. Fasulo, Ed., University of Florida, Gainesville, Fla, USA, 2006.