

Morphology and Three-Dimensional Reconstruction of the Digestive System of *Dermatophagoides farinae*

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Key Words

Alimentary canal • *Dermatophagoides farinae* • Digestive system • Morphology • Three-dimensional model • Visualization

Abstract

Background: The digestive system of *Dermatophagoides farinae* remains poorly documented. **Methods:** In this study, a three-dimensional (3-D) model of the alimentary canal of *D. farinae* was built for the first time based on hematoxylin-eosin staining, 76 cross-sections of the adult mite and 3-D reconstruction technology. **Results:** Spatially, the system included the prebuccal cavity, foregut, midgut, hindgut, anus and salivary gland. The prebuccal cavity was surrounded by gnathosoma; the foregut was composed of a muscular pharynx and an esophagus passing through the brain; the midgut was divided into an anterior midgut with a pair of ceca and a posterior midgut; the hindgut consisted of a relatively broad colon and a narrow rectum, and the salivary gland was irregular in shape and located in front of the brain. The 3-D images of the individual external surfaces of the reconstructed alimentary canal were displayed and the human-computer interaction was realized by performing arbitrary rotation and random sectioning. **Conclusions:** The 3-D model of the alimentary canal of *D. farinae* presented in this paper provides a stereoscopic view to study the adjacent relationship and arrangement of respective gut sections in the intact

mite. This study has implicit impact on morphology studies, immunohistochemistry, as well as in situ PCR and localization of the allergen in dust mites.

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Introduction

The house dust mite (HDM) *Dermatophagoides farinae* is a major source of inhalant allergens and can cause serious atopic diseases such as asthma and dermatitis [1]. According to a recent study, the localization of major allergens in HDM has been described by immunohistochemistry. It reveals that HDM allergens are mostly localized in the alimentary canal, gut contents and feces. The highest concentrations of Der p 1 have been found in the epithelium of the posterior end of the stomach [2]. Some Der p 1 is released through the salivary ducts behind the buccal cavity [3]. Der f 2 is synthesized in the epithelium of the anterior midgut and secreted in the lumen of the anterior midgut, mixing with digestive materials [4]. As such, there is obvious importance in studying the digestive system of *D. farinae*. However, the morphology of the mite is still poorly understood. Scanning electron microscopy has typically been used to examine interior organs of HDMs, such as the supracoxal glands [5], the peritrophic membrane [6], the digestive tract [6, 7] and the structure of the cuticle [8]. However, the obvious

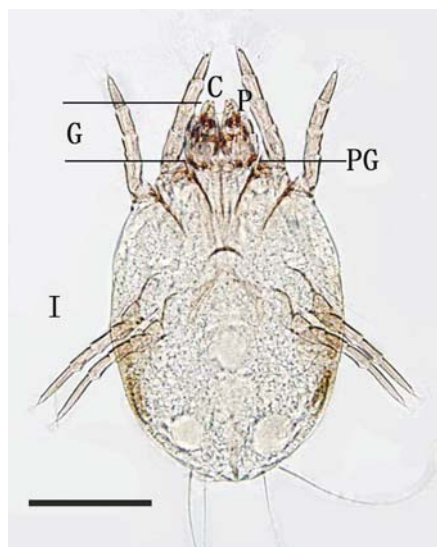


Fig. 1. Ventral view of the female adult *D. farinae* (×40). Scale bar = 50 μ m. C = Chelicerae; G = gnathosoma; I = idiosoma; P = palpus; PG = perignathosomal groove.

shortcoming of this technique is that it cannot provide detailed information about the three-dimensional (3-D) shape and size of the alimentary canal.

In this study, a 3-D model of the alimentary canal of the adult *D. farinae* was constructed based on computer software after the morphology of serial paraffin-embedded cross-sections was observed using light microscopy. The spatial position of different intestine sections was described in a stereoscopic view. Thus, this study provides a new approach to explore the inner structure of HDM and will give insight into the study of the digestive functions of HDM.

Materials and Methods

Preparation and Observation of Serial Paraffin Slices

Mites provided by the Allergic Disease Laboratory of Shenzhen University were used. Mites of *D. farinae* were maintained at 25°C and 75% relative humidity in incubators (LHL-113, ESPEC, Japan). Female mites were removed from the culture and fixed in Bouin's solution for 24 h. Because of their minute size and hard shell, we made the following improvement in the manufacturing process of paraffin slices compared with the traditional method: the fixed mites were soaked in 4% hydrochloric acid solution after treatment with phosphate-buffered solution for 30 min, and were then placed into a 1.5% agar solution and left to spontaneously coagulate. Subsequently, a clip was applied to the agar clump under an anatomical lens so that the insect's body was positioned in the center of the agar. After embedding in agar, the

clumps were successively dehydrated in graded ethanol and then in xylene before embedding in Paraplast (Sigma, USA). Serial 5- μ m sections were cut in a sledge macrotome and each slice was transferred to a glass slide coated with poly-L-lysine (Sigma) for drying on a hot plate at 60°C. After hematoxylin-eosin staining, sections were observed with a light microscope (BX51; Olympus, Japan) and images were transferred to a PC using a digital camera (DP 70; Olympus). The identification and terminology followed the description of Brody et al. [6].

3-D Reconstruction and Measurement of the Alimentary Canal

We selected a set of the aforementioned cross-sections prepared and photographed at ×600 magnification. The images were then treated using image analysis software Image-Pro Plus (Media Cybernetics, USA), which included brightness and contrast control, edge sharpening and image enhancement. In aligning two-dimensional (2-D) images, Fourier transform was used, primarily including the translation and rotation of a rigid body. The areas of the alimentary canal were excised and then overlaid so as to create a new image stack. By defining the x, y and z dimensions of the voxels that created an image volume, the 3-D model was synthesized and the spatial measurements such as distance, angle and cross-sectional area could be determined. Both 2-D and 3-D morphology parameters of the sections and the 3-D model were measured using the same software.

Results

Light-Microscopic Shape and Structure of the Digestive System

In this study, we found that the digestive system occupied large parts of the body cavity of the HDM, while the alimentary canal was tubiform.

Prebuccal Cavity. The prebuccal cavity, as the foremost part of the digestive system, was surrounded by gnathosoma located between the first pair of legs and connected with the idiosoma by the perignathosomal groove (fig. 1). The gnathosoma was composed of paired dorsal chelicerae and lateral pedipalps, an epistome and a hypostome. The musculature in chelicera provided a movable digit opening and closing action. In the cross-section, the labrum was triangular and fused with the epistome. The hypostome lay between paired pedipalps and its lateral surface fused with the pedipalpal coax (fig. 2). A dorsally projecting median ridge interrupted the hypostome and grew gradually along its full length to a Y-shape in the center of the hypostome. Two troughs between the hypostome and pedipalps were twisted to accommodate the movable digits.

Gut. The gut of *D. farinae* was divided into three main segments, foregut, midgut and hindgut, each of which could be subdivided into anterior and posterior regions. The foregut and hindgut were lined with cuticle. There

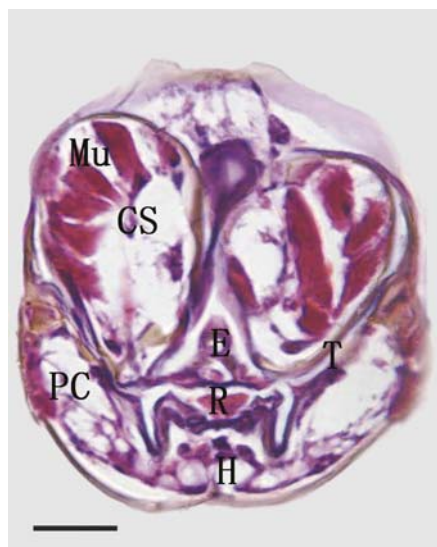


Fig. 2. Diagram of a cross-section of gnathosoma ($\times 100$). Scale bar = $10\ \mu\text{m}$. CS = Cheliceral shaft; E = epistome; H = hypostome; Mu = muscle; PC = palp coxa; R = ridge; T = trough.

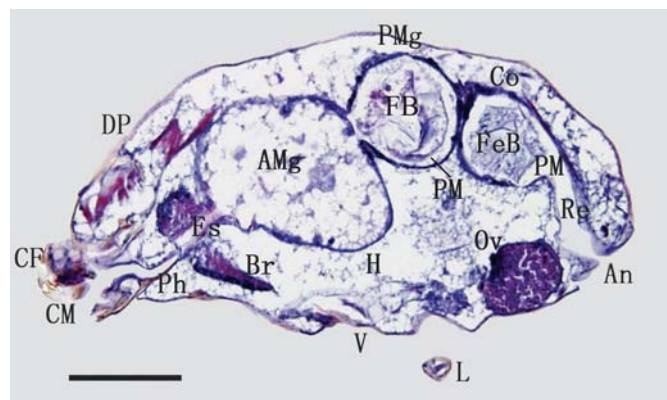


Fig. 3. Sketch map of a sagittal section through an adult female *D. farinae* showing the components of the alimentary canal ($\times 40$). Scale bar = $50\ \mu\text{m}$. AMg = Anterior midgut; An = anus; Br = brain; CF = fixed cheliceral digit; CM = movable cheliceral digit; Co = colon; DP = dorsal plate; Es = esophagus; FB = food ball; FeB = fecal ball; H = hemocoel; L = leg; Ov = ovum; Ph = pharynx; PM = peritrophic membrane; PMg = posterior midgut; Re = rectum; V = venter.

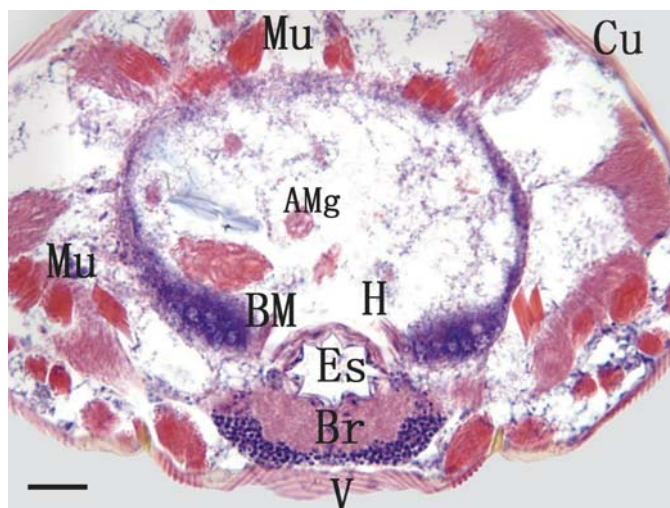


Fig. 4. Cross-section at the level where the esophagus (Es) protrudes into the anterior midgut (AMg) dorsal to the brain (Br; $\times 100$). Scale bar = $10\ \mu\text{m}$. BM = Basement membrane; Cu = cuticle; H = hemocoel; Mu = muscle; V = venter.

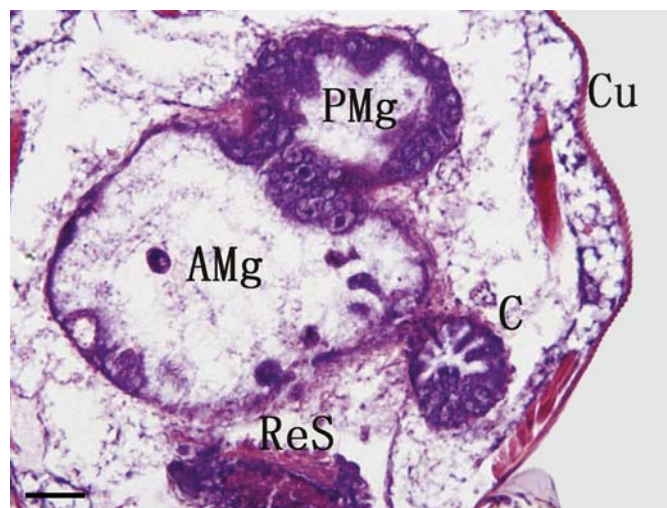


Fig. 5. Cross-section at the level where the anterior midgut (AMg) protrudes into the posterior midgut (PMg; $\times 100$). Scale bar = $10\ \mu\text{m}$. C = Cecum; Cu = cuticle; ReS = reproductive system.

was a constricted region between the anterior and posterior sections of the midgut. A similar constriction was also present at the junction of the midgut and hindgut (fig. 3).

Foregut. The foregut consisted of a muscular pharynx and an esophagus, which actively transferred food. Dorsal muscles originating from the hypostome were associ-

ated with the pharyngeal wall. In the sagittal section, the pharynx was arched towards the venter. The esophageal-pharyngeal junction, which was plicated, occurred near the base of the gnathosoma. The esophagus, lined by a cuticle of uniform thickness, extended dorsally through the nerve mass of the central nervous system and was

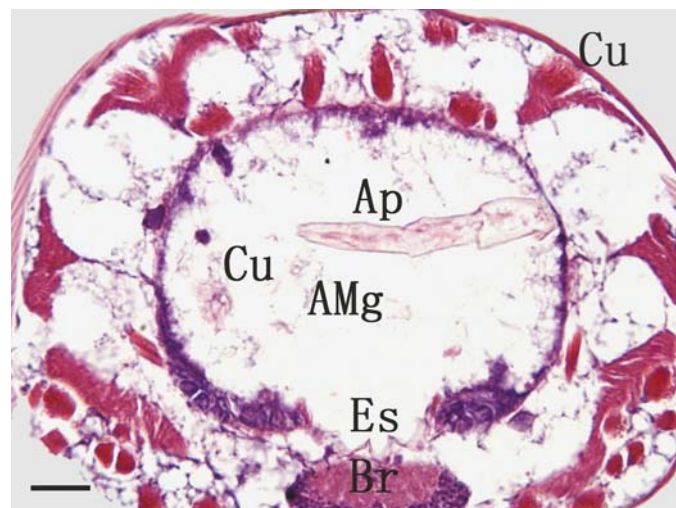
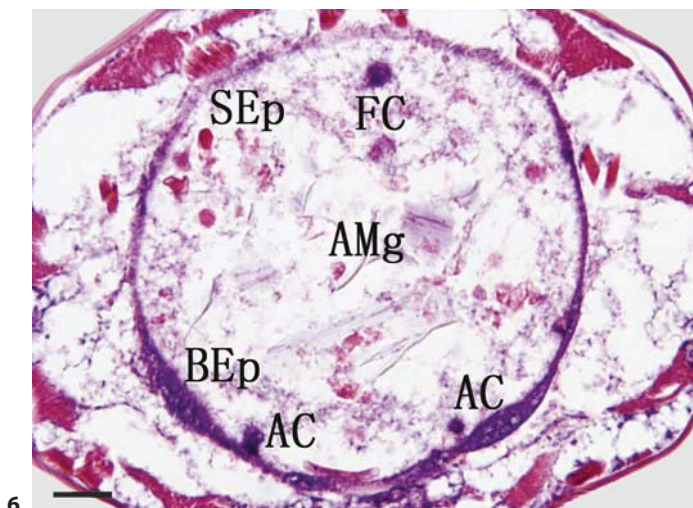


Fig. 6, 7. Diagrams of cross-sections of the anterior midgut (AMg; $\times 100$). Scale bar = 10 μm . AC = Attached cell; Ap = appendage; Br = brain; BEp = biggish epithelium; Cu = cuticle; Es = esophagus; FC = floating cell; SEp = smallish epithelium.

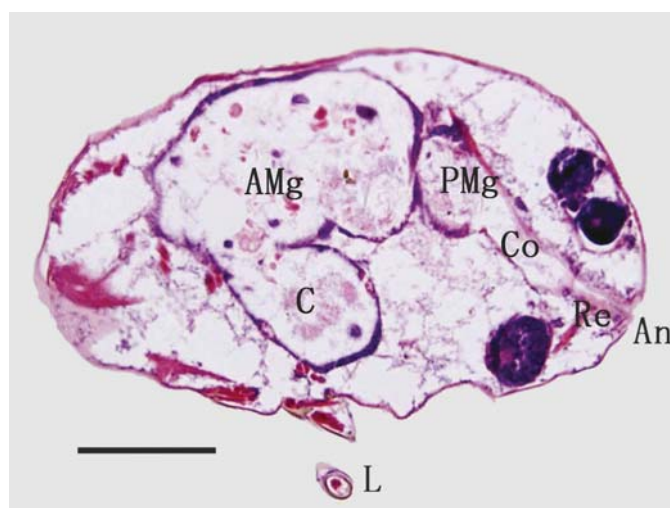


Fig. 8. Diagram of a longitudinal section representing the intumescent cecum (C; $\times 40$). Scale bar = 50 μm . AMg = Anterior midgut; An = anus; Co = colon; L = leg; Re = rectum; PMg = posterior midgut.

separated from it by an amorphous basement membrane (fig. 4). The cross-sectional shape of the esophagus was nearly an eight-pointed star and plicated, with the grooves formed between the plications becoming a portion of the hemocoel. The esophagus appeared dorsal to the brain and influxed to the midgut.

Midgut. The midgut of *D. farinae* was divided by a narrow opening into two sections: an anterior region and a posterior region (fig. 3, 5). The anterior midgut had a thinner wall and varied in size depending on the state of engorgement. The epithelium of the midgut had three kinds of cell types: cells near the esophagus of the anterior region, dorsal epithelial cells which were small and squamous, and ventral epithelial cells which were larger. In serial sections, the larger cells stretched to the gut lumen, some of which accreted the gut wall and were merely connected by a small part of the gut wall. Some cells floating freely in the gut lumen showed pleomorphism, which seemed to have originated from bigger cells (fig. 6). Digestive material in the gut lumen was loose and artus- es of other mites as well as portions of the cuticle were seen occasionally (fig. 7). Two ceca extended laterally from the anterior midgut (fig. 8, 9), and epithelial cells of its cavosurface were cuboid just like those in the posterior of foregut. Epithelial density of the ceca increased normally and the lumen of each was constricted, in which flocculent materials were often seen. The posterior of the midgut was globular, with squamous cells and cuboidal cells adhering to its wall. Obvious peritrophic-mem- brane-wrapped circular masses of food were separated from the gut wall by an unstained region to form fecal pellets. The posterior midgut connected the hindgut by another narrow opening (fig. 3).

Hindgut. The hindgut consisted of a colon and a rec- tum, which were both lined with cuticle. In the lumen,

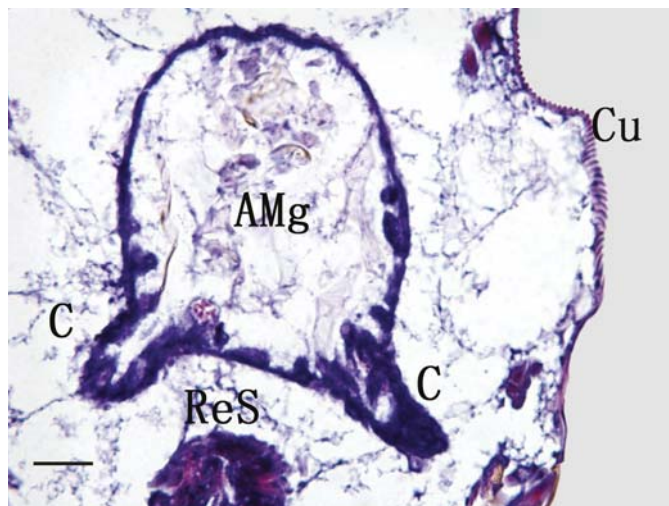


Fig. 9. Diagram of a cross-section of the anterior midgut (AMg) representing the contractile cecum (C; $\times 100$). Scale bar = 10 μm . Cu = Cuticle; ReS = reproductive system.

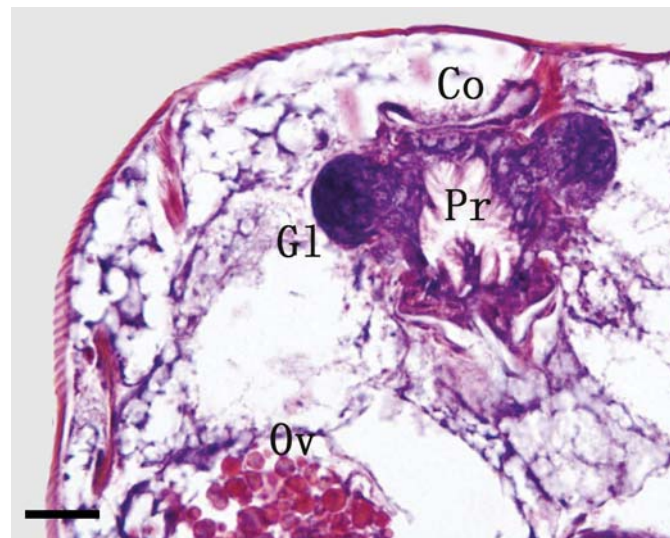


Fig. 10. Diagram of a cross-section of the colon (Co; $\times 100$). Scale bar = 10 μm . Gl = Gland; Ov = ovum; Pr = process.

fecal pellets wrapped by the peritrophic membrane were observed (fig. 3). Two apparent dorplices formed by the creases of the colon had a cell clump on each side, which was likely a glandular organ. The colon, in cross-section, appeared as indentations because of the many processes on it, and the cuticle was covered with a mucus-like lining (fig. 10). The rectum was a simple tube (fig. 8), terminating at a pair of anal plates.

Digestive Gland. The salivary gland located anterodorsally to the brain (fig. 11), which debouched to the pre-buccal space, stained darkly and appeared irregular.

3-D Reconstruction and Measurement

The integrity of the structure of the alimentary canal from serial sections is shown in figure 12a. The foregut, sloping dorsad, was a slender tube and the lacuna of the pharynx was narrow. The midgut was the most dilated portion, the anterior midgut occupied the bulk of the volume; the colon seemed dolichoglobular, which the lacuna narrowed gradually. The flat, tubiform rectum was connected to the slit-like anus.

Meanwhile, Image-Pro Plus provided a favorable window and the human-computer interaction was realized by performing an arbitrary rotation (fig. 12b) and random sectioning (fig. 12c), so as to observe the configuration, location and adjacent relationship of individual gut regions in the intact mite.

We summarized the data calculated from the area, perimeter and length of the gut region obtained from serial

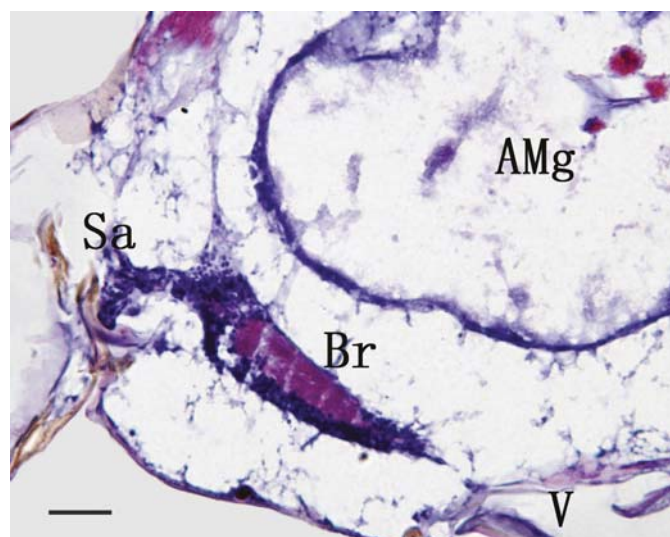


Fig. 11. Diagram of a longitudinal section representing the salivary gland (Sa; $\times 100$). Scale bar = 10 μm . AMg = Anterior midgut; Br = brain; V = venter.

sections, and statistical analyses were performed (table 1).

We tabulated the volume and length of each gut region of the alimentary canal from the reconstructed model after the model was sectioned (table 2).

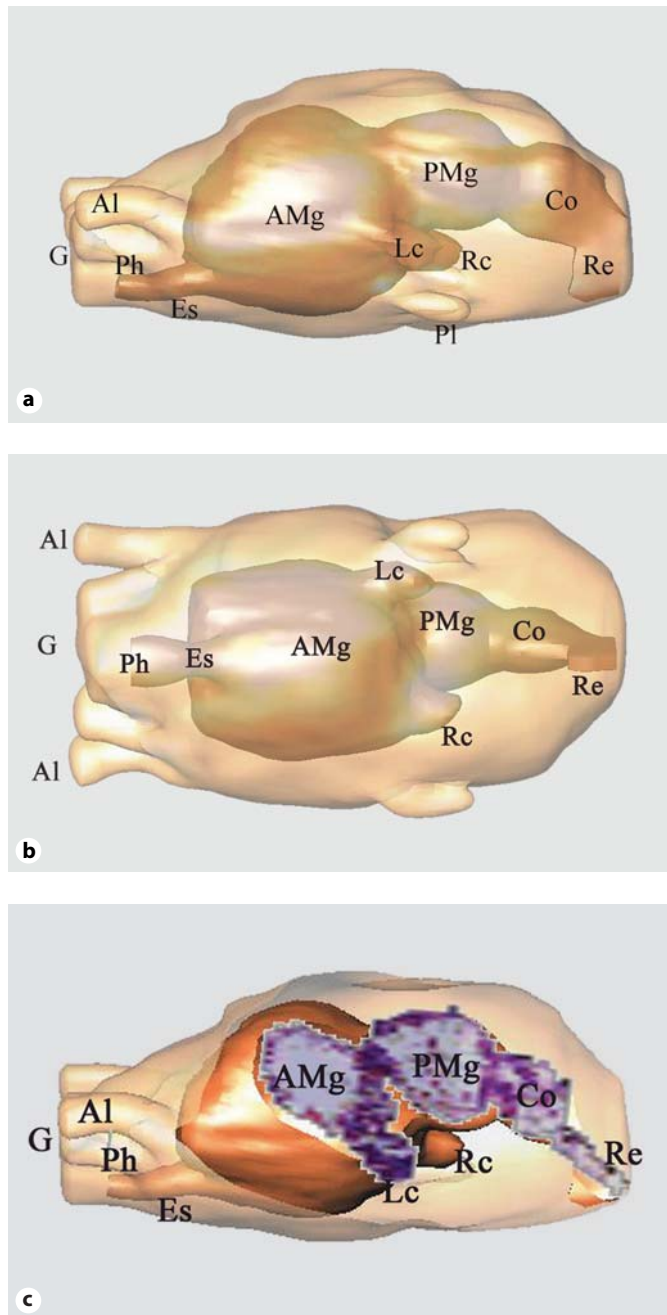


Fig. 12. Computer 3-D reconstruction of an adult female *D. farinae* (transparent mode of outside contours). **a** Left view. **b** Ventral view. **c** Random incision of the computer 3-D reconstruction model. Al = Anterior leg; AMg = anterior midgut; Co = colon; Es = esophagus; G = gnathosoma; Lc = left cecum; Ph = pharynx; Pl = posterior leg; PMg = posterior midgut; Rc = right cecum; Re = rectum.

Discussion

The anatomy of the intestinal structure of *D. farinae* is difficult to study because of its minute size. Traditionally, paraffin sectioning technology was often used to observe the digestive system, reproductive system and localization of allergens of *D. farinae* [9, 10], but it is difficult to ascertain the integrity of the slice as a result of the ectoskeleton of the body surface and 70–75% moisture capacity of the interior of the body [11]. Some modern technologies, such as the transmission and scanning electron microscope, require special equipment and a complicated process in material preparation. Furthermore, these technologies are also inconvenient for generating an intact view of the configuration. Improvements we have made include the novel approach of pre-embedding in agar before placing in Paraplast to maintain the integrity of the sections.

In addition, the nomenclature of the gut regions of Acari is confusing [6, 12, 13]. However, denomination of the pharynx and the esophagus is readily agreed upon. In this study, the structures of the foregut (pharynx and esophagus), midgut (anterior midgut, cecum and posterior midgut), hindgut and anus are named using the criterion of the presence or absence of a cuticular lining, following the terminology of Brody et al. [6]. Regarding the hindgut, in accordance with Vitzthum [14], the anterior and posterior structures are called the colon and rectum, respectively.

The foregut and the hindgut are both lined with cuticle. This lining probably prevents any significant absorption of food material. The antagonistic muscles of pharyngeal dilators and constrictors contracting reciprocally transfer food to the esophagus, showing a pumping function [15]. The plicated esophagus makes it possible to consume food of large size. Massive appendages and portions of cuticle are often seen in the gut lumen, indicating that *D. farinae* is cannibalistic. Moreover, the prebuccal cavity, pharynx and esophagus are capable of great distension.

The midgut has no cuticle and supplies the largest contact face. The anterior midgut, in particular, can distend to a sphere with a volume ratio of 77.58%, and serves as the dominant site of digestion and absorption. Several studies on the midgut epithelium of the Acari offer evidence of two digestive processes, extracellular and intracellular [16–18]. Studies with an electron microscope demonstrate that intracellular digestion takes place in cells, which at some time slough off into the gut lumen from the anterior midgut and are eventually decomposed

Table 1. Measurement of 2-D parameters of *D. farinae*

Parameter		Sectional area, μm^2				Thickness of the gut wall, μm
		gut tissue	gut lumen	gut region	abound	
Foregut	pharynx	6.81 ± 1.23	6.11 ± 2.04	12.92 ± 3.28	47.26 ± 17.68	0.63 ± 0.02
	esophagus	20.32 ± 4.65	80.15 ± 17.26	100.46 ± 18.80	207.24 ± 49.75	0.57 ± 0.09
Midgut	left cecum	229.44 ± 31.01	57.02 ± 20.95	286.46 ± 48.60	401.91 ± 92.12	5.33 ± 0.33
	right cecum	215.15 ± 55.89	46.85 ± 17.60	262.00 ± 46.04	376.47 ± 61.99	5.30 ± 1.28
Hindgut	colon	111.58 ± 56.98	551.59 ± 152.57	663.17 ± 167.11	942.67 ± 277.61	1.16 ± 0.57
	rectum	3.58 ± 0.86	1.13 ± 0.77	4.71 ± 2.15		0.63 ± 0.05

[6]. In this study, we found that some cells desquamate from the two larger cell rows in the venter of the anterior midgut and that cells floating in the gut lumen had possibly originated from those cells. In addition, to some degree, extracellular digestive processes exist in *D. farinae*. Considering the large size of food previously mentioned, mere intracellular digestion is not enough. Given that microscopy cannot observe their fine structure, this study was not able to detect secretory cells.

No difference was observed between the female and male *D. farinae* throughout the study through the examination of a considerable number of serial sections. It is noteworthy that minor differences observed in the size, location and morphology of cells of the midgut (especially the cecum) are attributable to differences in the degree of engorgement and stage of development. When the egg forms and moves forward, the size of the midgut grows downwards and the lacuna of the cecum becomes less obvious.

While the electron microscope method has previously been used to study species morphologically [19] and the composition of the *D. farinae* digestive system, little is known about its 3-D conformation and few data exist concerning its morphology parameters. 3-D reconstruction methods facilitate the assembly, alignment, analysis and visualization of serial sections. Today, the application of 3-D visualization has spread to nearly all tissues and organs of humans, but the number of 3-D reconstructions from serial biological tissue slice images are few, and neither a reconstructed model nor the complete data analysis of *D. farinae* have been presented. By generating a series of slices through the bulk sample and using computer processing when gut regions require highlighting, a complete 3-D image of the alimentary canal was created. It was found to be approximately tube like, varying according to the individual, and had constrict-

Table 2. Measurement of 3-D parameters of *D. farinae*

Parameter		Length μm	Volume μm^3	Volume ratio, %
Foregut	pharynx	18.34	5,876.62	0.33
	esophagus	41.86	16,822.70	0.95
Midgut	anterior midgut	142.38	1,380,791.58	77.58
	left cecum	38.15	14,623.77	0.82
	right cecum	40.88	11,497.76	0.65
	posterior midgut	75.92	244,756.79	13.75
Hindgut	colon	50.48	85,554.33	4.81
	rectum	50.12	19,707.85	1.11
Total			1,779,631.40	100

tions in the conjunction of gut regions. By measuring the morphologic parameters in 2-D and 3-D, we have gained insight into the true size and shape of the alimentary canal. On the other hand, with the help of immune electron microscopy, we may further explore the ultrastructural location of allergens in *D. farinae* and study the relationship between allergen secretion, release and diseases, providing guidance and reference for the development of allergen vaccines.

Acknowledgments

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References

- 1 Maunsell K, Wraith DG, Cunningham AM: Mites and house dust allergy in bronchial asthma. *Lancet* 1968;i:1267–1270.
- 2 Thomas B, Heap P, Carswell F: Ultrastructural localization of the allergen Der p I in the gut of the house dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Appl Immunol* 1991;94:365–367.
- 3 Rees JA, Carter J, Sibley P, Merrett TG: Localization of the major house dust mite allergen Der p I in the body of *Dermatophagoides pteronyssinus* by ImmunoStain. *Clin Exp Allergy* 1992;22:640–641.
- 4 Jeong KY, Lee IY, Ree HI, Hong CS, Yong TS: Localization of Der f 2 in the gut and fecal pellets of *Dermatophagoides farinae*. *Allergy* 2002;57:729–731.
- 5 Brody AR, McGrath JC, Wharton GW: *Dermatophagoides farinae*: the supracoxal glands. *J NY Entomol Soc* 1976;84:34–47.
- 6 Brody AR, McGrath JC, Wharton GW: *Dermatophagoides farinae*: the digestive system. *J NY Entomol Soc* 1972;80:152–177.
- 7 Tongu Y, Ishii A, Oh H: Ultrastructure of house-dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*. *Jpn J Sanit Zool* 1986;37:237–244.
- 8 Brody AR: Microscopic anatomy of the house-dust mite. *Proc North Central Branch Entomol Soc Am* 1971;26:64–65.
- 9 Walzl MG: Ultrastructure of the reproductive system of the house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* (Acari, Pyroglyphidae) with remarks on spermatogenesis and oogenesis. *Exp Appl Acarol* 1992;16:85–116.
- 10 Park GM, Lee SM, Lee IY, Ree HI, Kim KS, Hong CS, Yong TS: Localization of a major allergen, Der p 2, in the gut and faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2000;30:1293–1297.
- 11 Cui YB: The survey of biology, ecology and prevalence of dust mite. *Foreign Med Sci Parasit Dis* 2004;31:277–281.
- 12 Kuo JS, Nesbitt HH: The internal morphology and histology of adult *Caloglyphus mycophagus* (Megnin) Acarina: (Acaridae). *Can J Zool* 1970;48:505–518.
- 13 Coons LB: Fine structure of the digestive system of *Macrocheles muscaedomesticae* (Scopoli) (Acarina: Mesostigmata). *Int J Insect Morphol Embryol* 1977;7:137–153.
- 14 Vitzthum HG: Bronn's Klassen und Ordnungen des Tierreichs. Leipzig, Becker & Erler, 1940, p 480.
- 15 Johnston DE: A Comparative Study of the Mouthpart of the Mites of the Suborder Acaridei (Acari); PhD thesis. Columbus, Ohio State University, 1965.
- 16 Ludwig M, Alberti G: Ultrastructure and function of the midgut of camel spiders (Arachnida: Solifugae). *Zool Anz* 1992;228, 1–11.
- 17 Ludwig M, Alberti G: Fine structure of the midgut of *Prokoenenia wheeleri* (Arachnida: Palpigradi). *Zool Beitr* 1992;34:127–134.
- 18 Mothes U, Seitz KA: Functional microscopic anatomy of the digestive system of *Tetranychus urticae* (Acari: Tetranychidae). *Acarology* 1981;22:257–270.
- 19 Hart B J, Fain A: Morphological and biological studies of medically important house-dust mites. *Acarologia* 1988;29:285–295.