The Digestive System of *Diaphorina citri* and *Bactericera cockerelli* (Hemiptera: Psyllidae)

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ABSTRACT The psyllids *Diaphorina citri* (Kuwayama) and *Bactericera cockerelli* (Sulc) (Hemiptera: Psyllidae) are vectors of *Candidatus* Liberibacter spp., bacterial agents of serious agricultural diseases. The rapidly expanding geographical distributions of these diseases dictate increasing urgency for their control. Therefore, it is important to gain a full understanding of the psyllid digestive system in which the vector–pathogen interactions begin. Their midgut is looped so that the foregut–midgut and midgut–hindgut transitional regions are grafted together to form a composite tube within a filter chamber sheath. Unwanted sap components could thus be extracted directly into the hindgut, bypassing digestion. The esophageal lumen enters the chamber axially to become the inner midgut lumen. The upper half of this midgut section is bulbous while the lower half is tubular. The tube lumen exits the chamber to become the external midgut lumen, which loops through the hemocoel and reenters the chamber, becoming the inner hindgut lumen. The inner hindgut tracks the adherent inner midgut in an antiparallel direction. The composite tube is helically wound and undergoes one hairpin turn. The inner hindgut straps diagonally across the bulb and then exits the chamber next to the esophagus as the outer hindgut to anus. The source of honeydew, whether filtrate, midgut waste, or both, is questioned. Paired, spherical, primary salivary glands each have a digitate accessory gland and a lateral duct that leads to the stylets. The accessory gland lumen is lined exclusively with intima, whereas the primary gland apical cell membranes are indicated to be more complex.

KEY WORDS Psyllidae, alimentary canal, filter chamber, huanglongbing, Liberibacter

Citrus greening disease or huanglongbing (HLB) is considered the most threatening disease of citrus (*Citrus* spp.) worldwide, and it is the major limiting factor in citrus production in Asia and Africa (Capoor et al. 1974, Lallemand et al. 1986, da Graca 1991, Bové 2006, Browning et al. 2006). According to Roistacher (1996), affected citrus trees often survive only 5–8 yr and produce unusable fruit.

The disease is widespread in Asia, Africa, and the Saudi Arabian Peninsula. It was reported in July 2004 in São Paulo State, Brazil (Coletta-Filho et al. 2004, Teixeira et al. 2005), in the Caribbean Basin, and for the first time in the United States in south Miami-Dade County, FL, in August 2005 (Halbert 1998, 2005; Halbert and Manjunath 1994; Halbert et al. 2000; Halbert and Nuñez 2004).

Three species of phloem-limited bacteria are recognized: *Candidatus* Liberibacter asiaticus, *Candidatus* L. africanus, and *Candidatus* L. americanus (Teixeira et al. 2005). To date only *Ca.* L. asiaticus has been identified in Florida (Halbert 2005), however, future introductions of additional species of *Ca.* Liberibacter and of the psyllid vector are considered likely.

The establishment of *Ca.* Liberibacter asiaticus in Florida has greatly increased the significance of the presence of the psyllid *Diaphorina citri* (Kuwayama) there (Halbert 1998) and elsewhere and underscores the urgent need for basic knowledge about vector–pathogen interactions that is now essential for devising effective management strategies to deter or block vector-mediated pathogen transmission.

The psyllid *Bactericera cockerelli* (Sulc), a relative of *D. citri*, is a major pest of potato (*Solanum* spp.), tomato (*Solanum* spp.), and other fruiting vegetables and is the vector of a newly described disease of tomato and potato caused by *Ca.* Liberibacter psyllaurous (Hansen et al. 2008). However, most aspects of acquisition and transmission of *Ca.* L. psyllaurous by the psyllid are unstudied (Hansen et al. 2008).

Concise descriptions of psyllid vector digestive systems are needed to understand the transmission pathway of psyllid-borne pathogens, from ingestion, to passage through the consecutive internal organs, and during transmission to the subsequent host plant. The only information available regarding the organization of the psyllid digestive system comes from classical studies conducted 80–90 yr ago [Dufor (1833) for *Psylla ficus*, Witlaczil (1885) for *Psylla buxi* (L.) and *Trioza urticae* (L.), Macloskie (1886) and Packard 1898 for *Psyllopsis* sp., Saunders (1921) for *Psylla mali* Schmidberger]. Brittain's copy (Brittain 1923) of

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Saunders' drawing of the *P. mali* gut made its way into the secondary literature as representative of the family (Pesson 1951, Goodchild 1966, Weber 1968, Bielenin 1993). Saunders noted an eccentric lobe on the salivary gland that he did not identify. More recently, Ullman and McLean (1986) studied *Psylla pyricola* Förster with modern techniques but concentrated on sensilla of the oral region only.

The purpose of this study was to elucidate the construction of the digestive system of *D. citri* and *B. cockerelli* so that its components can be recognized and identified at the ultrastructural level during transmission pathway studies. Particular emphasis was placed on the alimentary canal component known as the "filter chamber," because it was anticipated to have great structural complexity and to be the anteriormost organ that lacks an inner cuticle. If the canal anterior to it is fully lined with a pathogen-impenetrable cuticle, then the filter chamber would be the first organ of opportunity encountered in the earliest stages of *Ca.* Liberibacter transmission.

Materials and Methods

Adult *D. citri* were collected at Southwest Florida Research and Education Center, University of Florida, in Immokalee, FL, from colonies reared on *Citrus* spp. or *Murraya paniculata* (L.) (mock orange jasmine) or from the field on *Citrus* spp. Adult *B. cockerelli* were collected from infested tomato plants grown in a greenhouse in southern Arizona.

Psyllids were dissected ($n = \approx 60$ each) in a weak solution of toluidine blue and 0.1 M Na-K phosphate saline buffer (PBS), pH 7.75, to remove the alimentary canal and salivary glands. Gentle pressure was exerted under a dissecting microscope to break extirpated filter organs open and study their internal conduit system. For scanning and transmission electron microscopy (SEM and TEM, respectively), extirpated organs were transferred to 4% formaldehyde, 0.5% glutaraldehyde in PBS for a 2-h fixation; rinsed two times 15 min in PBS; and dehydrated in a graded ethanol series. Organs relegated to TEM and serial reconstruction were embedded in LR White resin (25% resin:75% ethanol for 1 h, 75% resin:25% ethanol for 1 h, 2× 100% for 1 h, polymerized overnight at 55°C), and sectioned and stained with uranyl acetate/lead citrate. Organs relegated to SEM were mounted whole or freeze fractured with a razor blade on a metal block in liquid nitrogen and then critical point-dried and mounted.

Once the configurations and external boundaries of these organs were determined, whole specimens were processed for TEM by gluing to a thumbtack in a micro-watchglass, submerging them in PBS, removing air bubbles with Aerosol OT [sodium 1,2-bis (2-ethylhexoxycarbonyl) ethane sulfonate], rinsing, and flooding with fixative. Specimens were then opened with separate, transverse cuts across the vertex, terminal abdominal segment, and meso-metathoracic juncture by using microscissors. Specimens were fixed overnight, rinsed, dehydrated, and embedded in LR White. Blocks were cut with an LKB Ultratome 5 and viewed under JOEL 100CX and Philips CM12 transmission electron microscopes.

Results

Alimentary Canal. *General Anatomical Organization.* The alimentary canal of *D. citri* and *B. cockerelli* showed no differences in gross anatomical organization (Fig. 2B and C). Both were modified from the general insect canal (Fig. 1A) as follows. The midgut (ventriculus) was looped so that its anterior and posterior ends were in proximity to each other and to the esophagus (Fig. 1B and C, a) and hindgut (Fig. 1B and C, b), which, by their enclosure inside a *sheath* (coined by Kershaw 1913) (Fig. 1B and C, c), formed the filter chamber complex. Within the complex, the midgut component (Fig. 1B and C, d), and what is herein provisionally (see Discussion) termed the *inner hindgut* component (Fig. 1B and C, e), were longitudinally adherent to each other, forming a composite tube. The inner midgut section was divided into two parts, an upper bulbous section and a lower tubular section. The external midgut was divided into two parts also, an inflated anterior part (Fig. 1C, f) and a tubular posterior part (Fig. 1B and C, g), the latter providing the looping back to the point of reentry into the filter chamber. Three ventricular sections are identified in this basic design, termed V_1 (the inner midgut), V_2 (the outer, inflated midgut section), and V_3 (the outer, tubular, midgut section) following the ordinal level diagram devised by Snodgrass (1935, p. 384, fig. 209A) (Fig. 1D). The lumen of all three is the same continuous lumen that starts at the mouth and ends at the anus.

Orientation. The hemocoelic positions of the alimentary canal components were the same for both species, as follows. The esophagus extended from the mouth posteriorly through the thorax, whereas the hindgut extended from the anus anteriorly through the abdomen. The distal extremities met at the base of the abdomen where they were attached to the anatomical apex of the filter chamber. Although confined to the abdominal base by these ectodermal tubes, the filter chamber was free to twist and turn without regard to dorsoventral or left–right orientation.

The abdominal base was walled anteriorly by the thoracic musculature and posteriorly by adipose tissue and gonads in the abdomen, loosely confining the midgut loop to this region also. Tracheal attachments confined the midgut loop and its appendages (Fig. 1E, q and F, p) to this region, too; but the location of attachments were variable from specimen to specimen, allowing for some latitude in the orientation of the loop. In some specimens, the loop was twisted and convoluted within the base of the abdomen, whereas in others, sections of the loop were located deeper into the thorax and abdomen.

When extirpated alimentary canals were allowed to spread in the dissection buffer, they settled into a free-floating configuration with the least tension between its components. This configuration was used

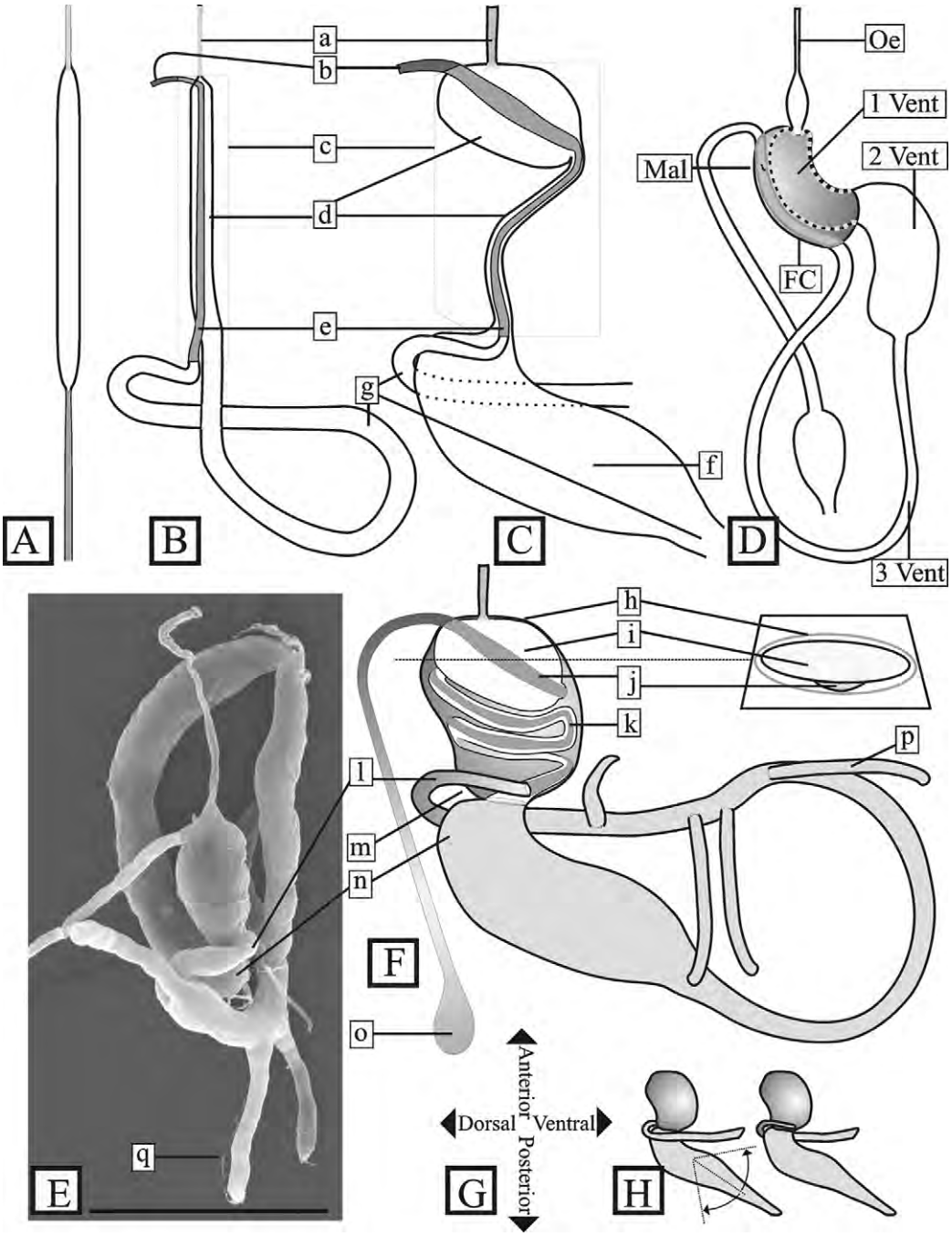

Fig. 1. Configuration of the *D. citri* and *B. cockerelli* alimentary canals. (A–C) Stepwise schematics. See text for narrative. (D) Schematic of a generalized homopteran alimentary canal possessing a filter chamber. Redrawn from Snodgrass 1935, p. 384, fig. 209A. Original labeling is retained as needed. (E) Scanning electron micrograph of an extirpated canal of *B. cockerelli*. Line = 300 μm. (F) General configuration of the *D. citri* and *B. cockerelli* alimentary canal. Inset, cross section through indicated plane. (G) Assigned orientation of the alimentary canal of both species. Orientation arrows apply to this schematic only, not the micrograph in E. (H) Right- and left-handedness with regard to the attachment of the midgut arm to the waist was noted in both species. The second ventricular section (V_2) can flex at an ≈90° angle. Oe, esophagus. 1, 2 & 3 Vent, first, second, and third midgut (ventricular) sections, corresponding to V_1, V_2, and V_3 in this article. FC, filter chamber sheath. Mal, malpighian tubules. a, esophagus. b, external hindgut. c, box surrounds those sections that are enclosed by the filter chamber sheath. d, first ventricular section (V_1) (white). Upper V_1 is a bulb, whereas lower V_1 is a tube. e, inner hindgut (gray) is fused to V_1 and tracks its length, strapping itself diagonally across the bulb before it exits as the external hindgut. f, second ventricular section (V_2). g, third ventricular section, the midgut loop (V_3). h, filter chamber sheath; loosely drawn

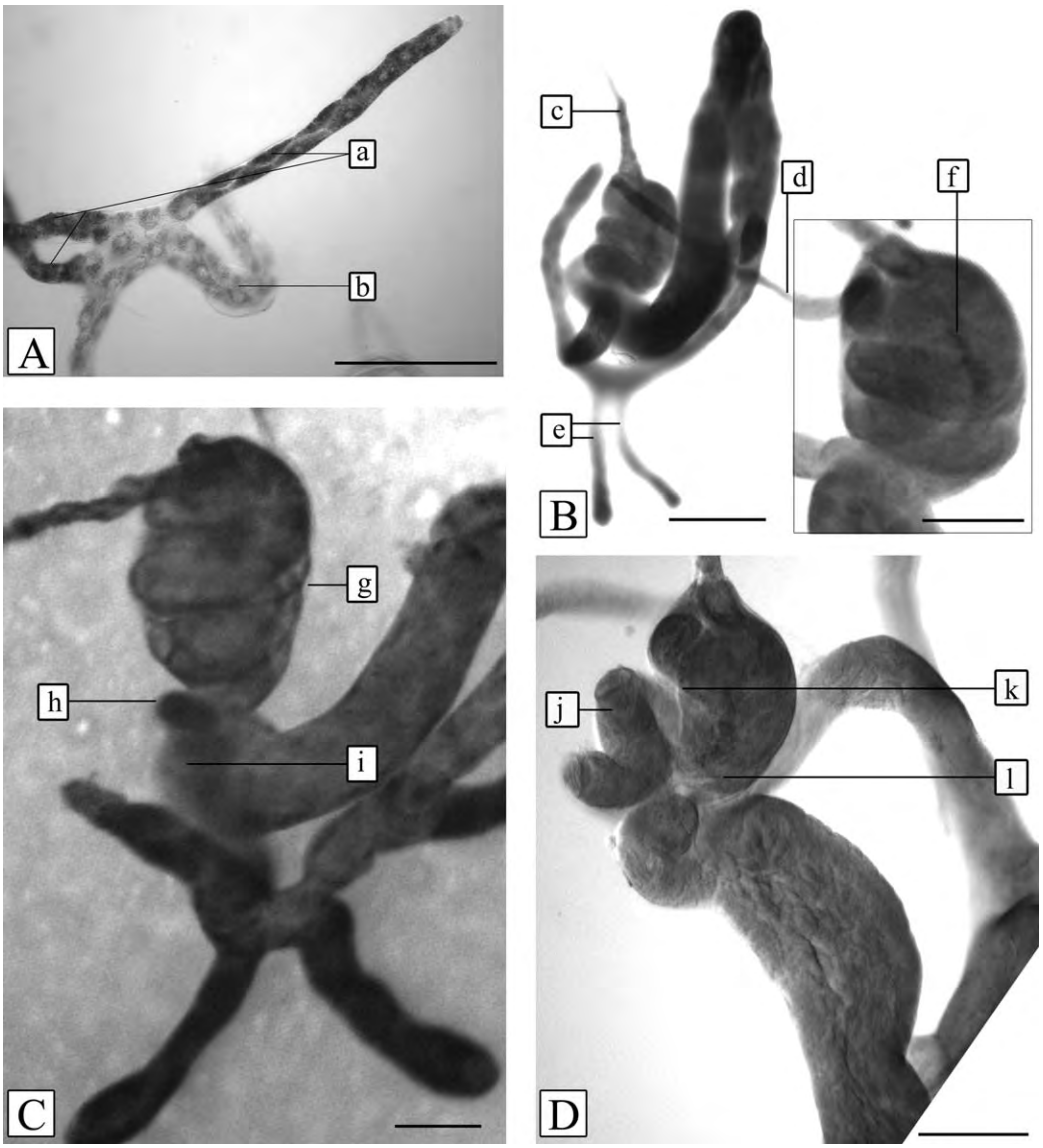

Fig. 2. Light micrographs of the *B. cockerelli* and *D. citri* alimentary canal components. (A) *B. cockerelli* canal showing characteristic rhomboid cells with transparent pericytoplasmic zones. Third ventricular (V_3) appendages are brown or green in both species (J. Castillo, personal communication). (B) *D. citri* alimentary canal. Inset, close-up of a *D. citri* filter chamber showing the bulb lumen. This lumen shows through faintly in other plates of this figure. (C) *B. cockerelli* alimentary canal. D) *D. citri* filter chamber broken open to show how the hairpin turn seats against the V_1 bulb. a, V_3 appendages. b, V_3. c, esophagus. d, external hindgut. e, second and third midgut appendages are characteristically closely set in both *B. cockerelli* and *D. citri*. f, lumen of the V_1 bulb. g, sheath. h, midgut arm. i, V_2. j, hairpin turn of lower V_1 broken free of the sheath to show how it is seated against the venter of the bulbous upper V_1. k, impression of the hairpin turn made on the underside of the V_1 bulb. l, posterior limit of the bulb in this extirpated specimen extends well beyond the hairpin turn. Lines = 500 μm.

Fig. 1. (continued). for clarity. Normally, sheath tightly envelopes the internal filter organ. Cross-sectional inset shows the topical attachment of the crescent-shaped inner hindgut to the spherical-shaped V_1 to form the composite tube. i, upper V_1 bulb. j, inner hindgut. k, hairpin turn of the composite tube. l, midgut arm tracks externally along the waist and penetrates into the filter chamber venter. m, waist. The dorsal interior of the chamber in the waist is vacant of conduit. n, dorsal lobe of V_2. o, anal bulb. p, first (anterior-most) of four midgut appendages. q, tracheae attached to the third ventricular appendage. The first ventricular appendage is behind the filter chamber.

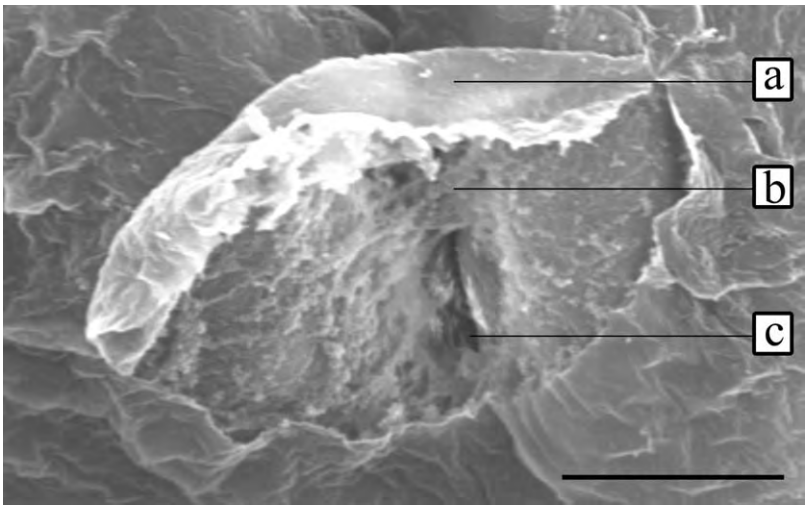

Fig. 3. SEM of a *B. cockerelli* filter chamber with a rip in the sheath, showing internal helical conduits and connective material that seems to hold the coils in place. (a) Rip in sheath. (b) Connective tissue. (c) Space lateral to contact of helical coils. Line = 20 μm.

to establish convention for references to orientation (Fig. 1G). In situ orientation in live specimens was distorted from the latter configuration, due to pressure exerted by other neighboring organ systems and endoskeletal features. Also, left- and right-handedness was discovered in the attachment of the distal midgut (Fig. 1H). Therefore, only anterior, posterior, dorsal and ventral aspects can be defined by this approach.

Detailed Anatomical Organization. The following detail was determined to be true for both species except as noted. The sheath (Fig. 1F, h) was a thin envelope tightly enclosing the internal filter organs, with apparently no openings that would allow for direct continuity between the blood and the interior. No clues were found to indicate which embryological tissue type the sheath is derived from or composed of. The upper V_1, located in the anterior one third of the filter chamber, was a more or less hemispherical bulb with a diagonally flat posterior face (Fig. 1 F, i). Lower V_1 was a narrow, helically coiled tube with a single hairpin turn (Fig. 1F, k) that extended posteriorly for the rest of the filter chamber's length. In some extirpated specimens, the bulb was expanded further posterior than the hairpin turn (compare Fig. 1F, i with Fig. 2D, l). A conspicuous constriction in the contour of the alimentary canal, herein referred to as the *waist* (Fig. 1F, m), was present where V_1 left the filter chamber as V_2, in a ventroposterior direction, at an $\approx$110° angle to the long axis of the filter chamber. V_2 was elongate, bulbous, with a strong *dorsal lobe* (Fig. 1F, n), and its radius gradually decreased as it neared V_3, the midgut loop. Most notable in extirpated specimens was the settling of the distal section of the V_3 loop (the *midgut arm*, Fig. 1E and F, l) into a horizontal attitude because of its histological attachment to the point of reentry. It always crossed the full width of the waist, closely tracking the latter's left or right transverse constriction, before entering into the ventroposterior left or right side of the filter chamber (Fig. 1H).

The continuous lumen of the midgut arm reentered the filter chamber at the waist to become the lumen of the inner hindgut. The inner hindgut joined immediately in adherence with lower V_1, and, as a composite tube, both pressed against the venter of the waist. The waist dorsal to this was vacant of tissue allowing the filter chamber to flex at approximately a 90° angle about V_2 (Fig. 1H).

The inner hindgut, in adherence to V_1, tracked the coiled shape of V_1 in an antiparallel direction and then crossed upper V_1 diagonally (Fig. 1F, j) and exited the chamber apex next to the esophagus. Relative to the long axis of the variably positioned filter chamber, the esophagus met the filter chamber axially, whereas the external hindgut left the filter chamber tangentially, in line with the inner hindgut that preceded it. The external hindgut continued posteriorly, ending as a bulb at the anus (Fig. 1F, o).

Four $\approx$0.3-mm-long appendages extended from V_3 in the last third of its length (Fig. 1F, p). The basalmost appendage pointed ventrally, the second and third pointed posteriorly, and the fourth pointed laterally. The attachments of the second and third were separated by $\approx 1\times$ their basal girth, whereas the other two were spaced further apart. Numerous dissections showed that the apices of these appendages were sites for transient attachment/detachment/reattachment of tracheae (Fig. 1E, q).

In both species, the unstained filter chamber was colorless and hyaline. Cells of V_2 and V_3 were opaque white, whereas those of the V_3 appendages were dark green or brown (J. Castillo, personal communication). Cells of V_2, V_3, and V_3 appendages had a transparent pericytoplasm, giving each cell a rhomboid appearance (Fig. 2A, a and b).

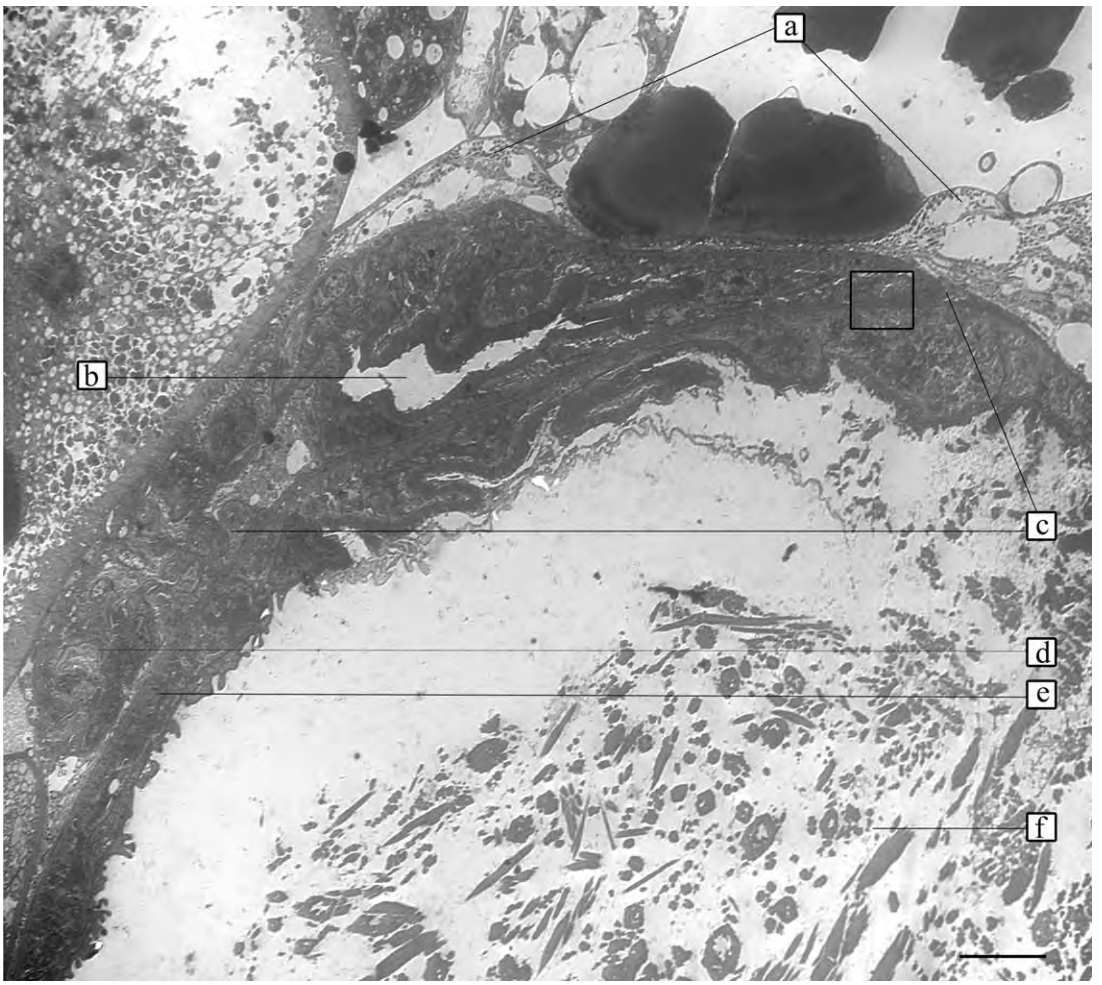

Fig. 4. Ultrastructure of the *B. cockerelli* filter organ. Cross-section passes through upper V_1. Inset, coappressed basal lamina of the inner hindgut epithelium and the upper V_1 epithelium, magnified in Fig. 5.(a) Putative sheath. (b) Lumen of the inner hindgut. (c) Lateral tapers of the inner hindgut. (d) Intervening material obfuscating true end of left lateral taper in c (cf. Figure 1F, inset). (e) Upper V_1 epithelium. (f) Upper V_1 lumen. Line = 10 μm.

Internal Complexity. The sheath of both species could be peeled away to expose an interstitial space between the composite tube coils, indicating that the sheath was a separate component from the internal conduit system (Fig. 3). SEM of the *B. cockerelli* chamber indicated that the space between conduit loops was laced with a web of material that seemed to hold them in place. The contents of the interstitial chamber space, be it gas, fluid, mucilage, or some other material, were not determined.

In all cross sections of both species studied, the inner hindgut maintained a convex, crescent shape, and a topical adherence to the circular shape of V_1 (Figs. 1F, inset; 4, and 5). This configuration, and their respective lumina, allowed for distinguishing them from each other and from other organs (Fig. 6). The adherence of the two organs was a planar coalescence of the two opposing basal lamina (Fig. 5a) with crescent-shaped involutions on either side (Fig. 5, b and c). Also, there were no indications of malpighian tissue inside the filter chamber.

Because of the potential for processing artifacts, the degree of inflation and torpidity of the composite tube components cannot be considered valid observations. However, it was noted that the coils of lower V_1 were pressed up against the flat plane of the bulb apex, against each other, and against the inner filter chamber wall (Fig. 2B–D). The center of the chamber was not occupied by any axial, supportive tissues (Fig. 2D) and seemed to provide space for distension of the tube during feeding. In Fig. 1F, lower V_1 is drawn with space between the coils for clarity.

Salivary Glands. Both species had one pair of primary salivary glands (PSGs) and one pair of accessory salivary glands (ASGs) located directly dorsal to the procoxae (Fig. 7, inset). PSGs of specimens fixed in their natural state and processed for TEM were observed to be irregular polygons rather than spheres

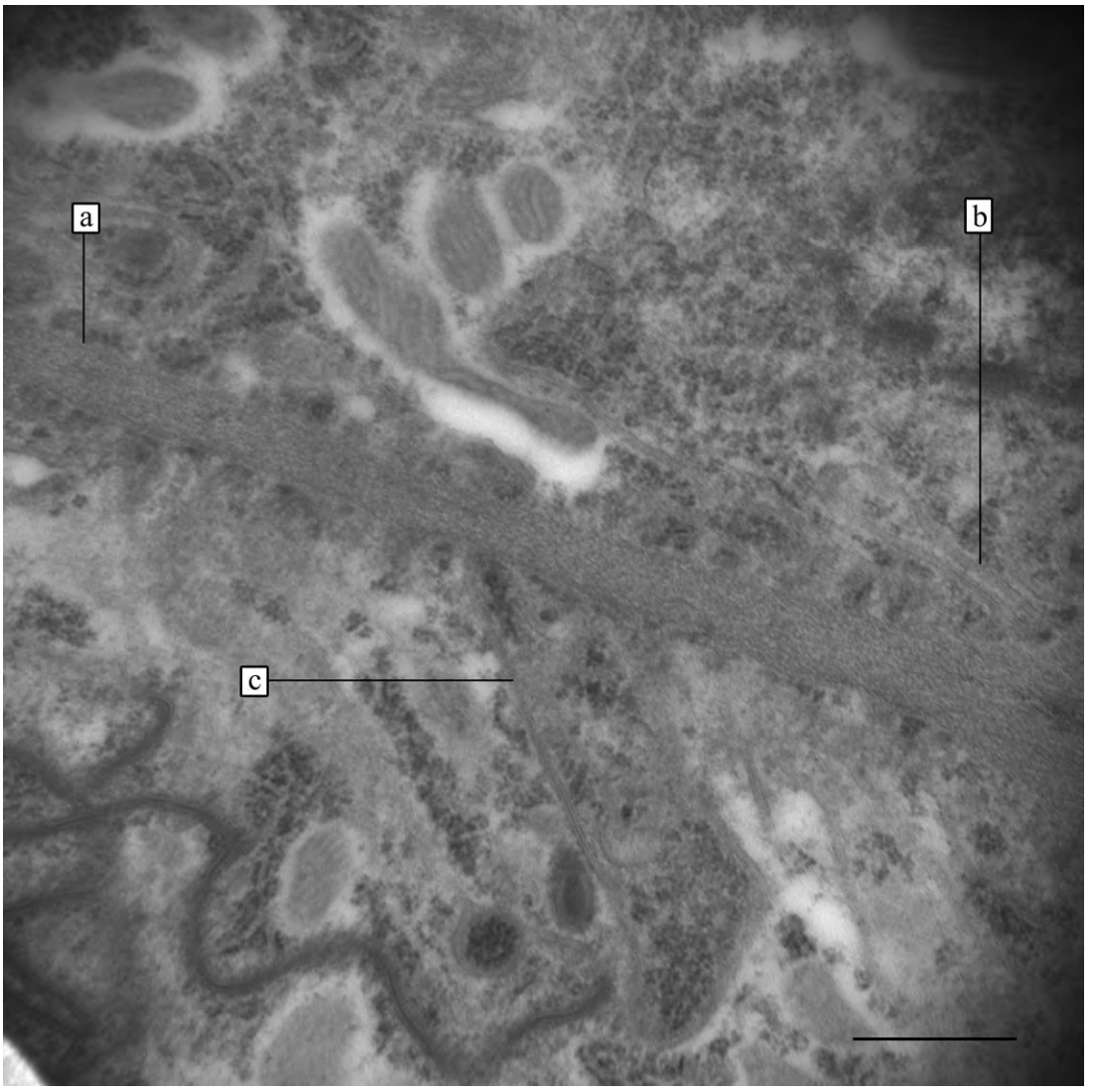

Fig. 5. *B. cockerelli* filter organ. Inset from Fig. 4. Close-up of the interface between the inner hindgut epithelium, above, and the upper V_1 epithelium, below. (a) Coappressed basal lamina. (b) Involutions radiating into the inner hindgut cytosol. c) Involutions radiating into the upper V_1 cytosol. Line = 0.5 μm.

because of pressure from neighboring organs and endoskeletal features. ASGs were variously longitudinally folded so that multiple cross sections of its canal showed up in some micrographs.

The PSGs of both species were connected to a strongly sclerotized, box-shaped, oral complex by their respective *salivary gland ducts* (Figs. 7e and 8a). The apical cells of the salivary gland duct were embedded in the cells of the anterior PSG (Fig. 11h). By definition, the location of the duct's connection determines the anterior face of the PSG. The ASG was joined to the PSG at a point adjacent to this connection. In both species, the beak was located between the closely approximated prothoracic legs, leaving very little space internally between the oral box and the PSG (Fig. 7, inset). Correspondingly, the two salivary gland ducts were extremely short, ≈50 μm. In *D. citri*, the existence of a common duct that the two lateral ducts merge into is indicated by the single salivary channel observed in the stylets. Sectioning came close to this junction but did not reveal its exact luminal configuration because of its extremely short length, estimated to be on the order of 5u or less (Fig. 8d). The basal ducal configuration of *B. cockerelli* was not investigated.

The extirpated PSG was spherical, ≈100 μm in diameter, but slightly flattened anteriorly. The flat anterior was conferred by approximately four to five small, squamous cells (Fig. 7f), whereas the spherical shape was conferred by ≈10 large, wedge-shaped semicircular cells (Fig. 7g). The term *hesperidiform* is herein coined to describe such a spherical organ com-

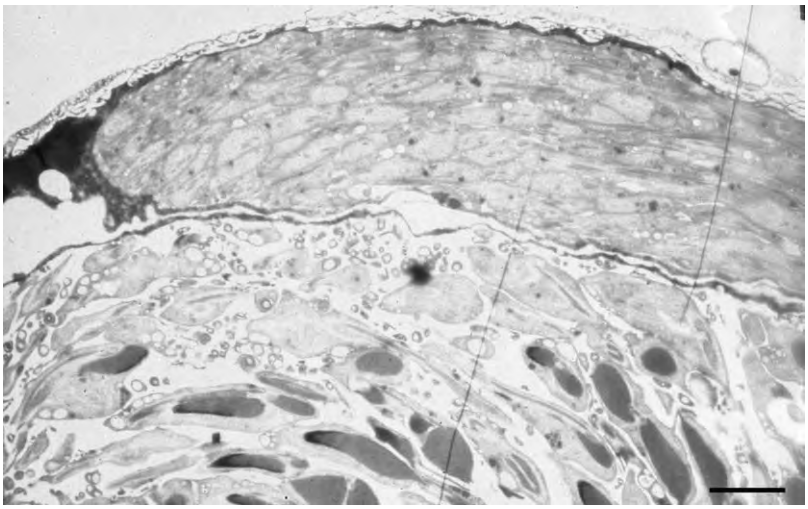

Fig. 6. *B. cockerelli* male gonad. This is the only other bulbous organ that might be confused with the filter chamber. Line = 4 μm.

posed of wedge-shaped cells resembling sections of *Citrus* spp. fruit. Light microscopical examination of all dissections of both species revealed a funnel-shaped pattern to the PSGs, indicated in TEM also (Fig. 11, inset). Apicomesal cells had strongly staining material in their cytoplasm, apicolateral cells had weakly staining material in their cytoplasm, but basal cells did not stain appreciably. TEM corroborated these observa-

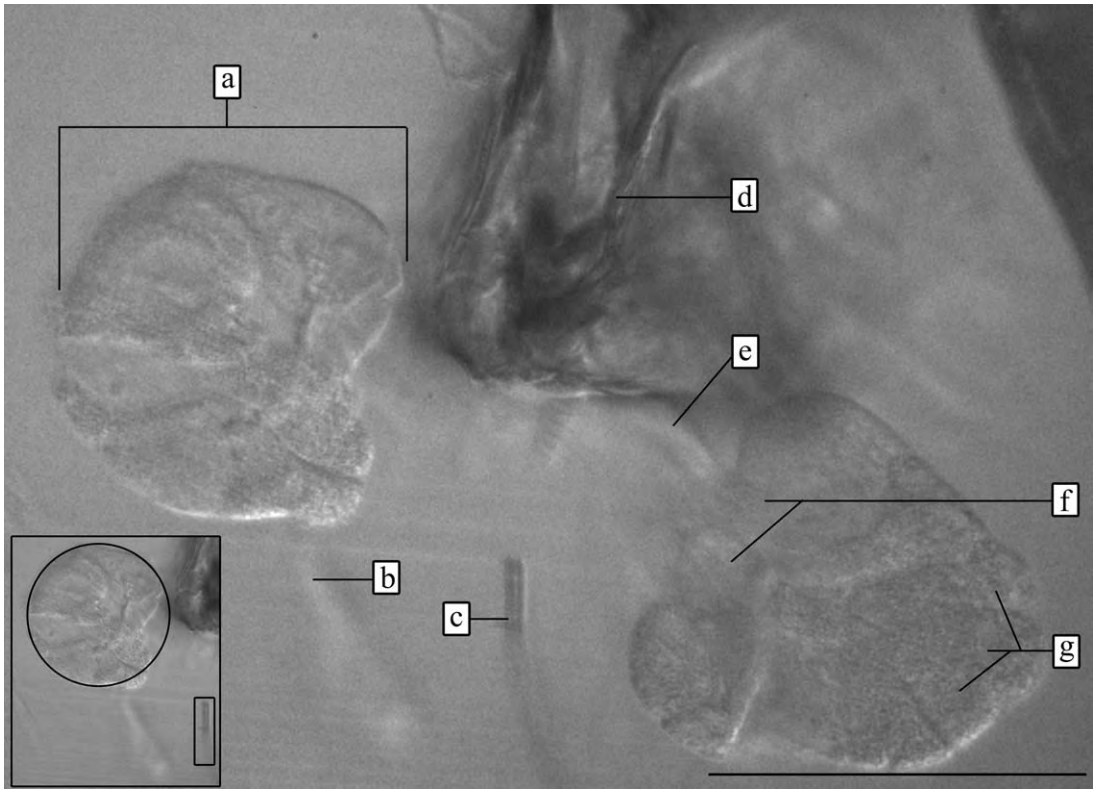

Fig. 7. Block face of embedded *D. citri* oral complex, ventral view. Inset, circle represents the procoxal cavity. The PSG is located directly dorsal to it. Rectangle surrounds the stylet bundle. (a) PSG. (b) ASG slightly out of focus. (c) Stylet. (d) Anteclypeus. (e) External salivary duct. (f) Anterior squamous cells. (g) Posterior wedge-shaped cells, giving the PSG a "hesperidiform" or *Citrus*-like appearance. Line = 100 μm.

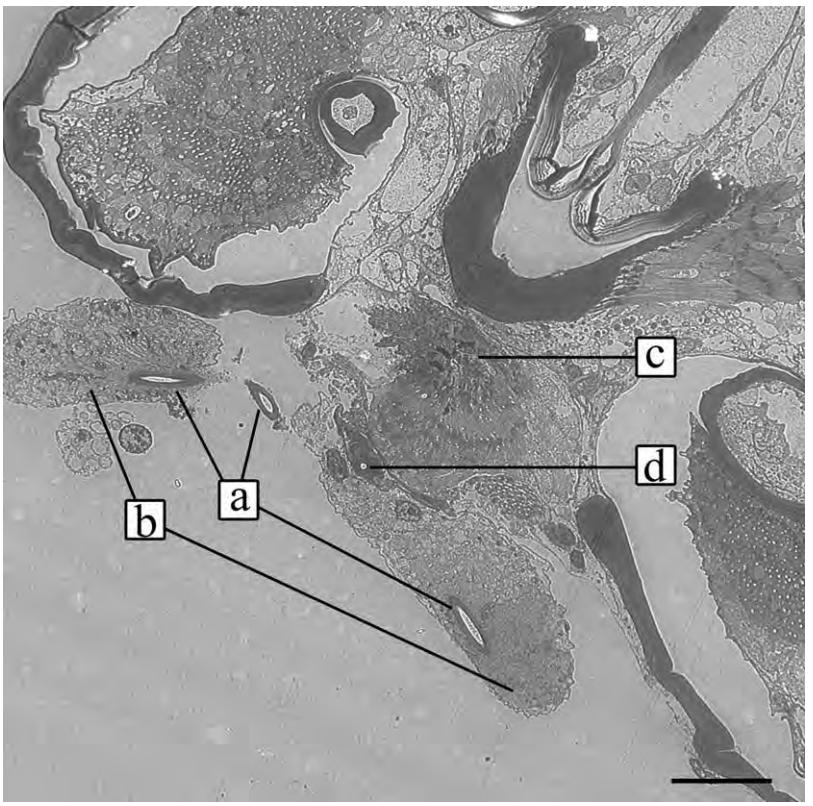

Fig. 8. Cross section of embedded *D. citri* oral complex, through the block in Fig. 7. (a) Left and right lateral salivary gland ducts. (b) Salivary gland duct cells. (c) Salivary syringe muscle complex. (d) Intima lined lumen in the location expected for a very short common duct. It is expected that the lateral ducts merge into a common duct in this area. Line = 100 μm.

tions: Apicomesal PSG cells contained full, or nearly full, electron opaque spherules (Figs. 9a, 10a, and 11a), and apicolateral cells contained what seemed to be remnants of spherules (Figs. 9c and 11, b and d). A third cell type, adjacent to these two types, had electron transparent spherules (Figs. 9b and 11c). Basal cells had no indication of secretory capacity (Fig. 11e). Storage material in full spherules was highly organized into concentric arrays of densely packed subunits that were beadshaped in cross section (Fig. 10) and columnar in longitudinal section. Different degrees of mobilization, whether importation or exportation, of storage material were apparent from spherule to spherule (Fig. 10a). Further characterization of this transport system is underway.

The ASG was a short, stout, digitate, slightly clavate appendage of the PSG, about as long as the PSG diameter in both species (Fig. 11). The connection of the ASG to the PSG was not by a long thin, tether, instead the ASG was broadly connected to the PSG

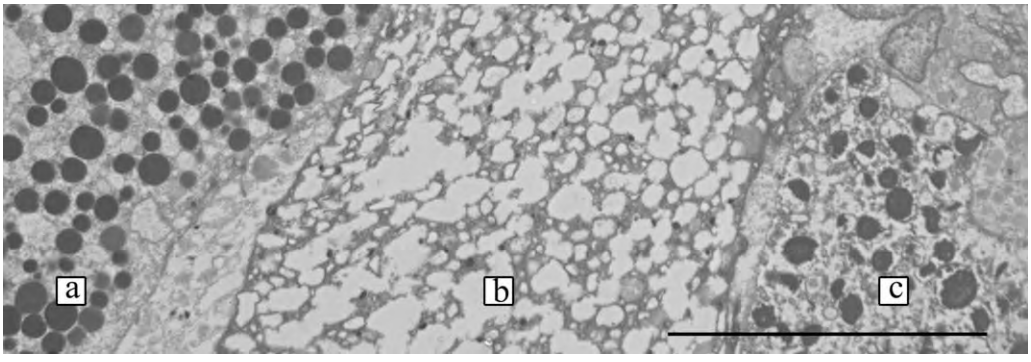

Fig. 9. *D. citri*. Transverse, mesal cross section of a PSG from the block in Fig. 7. (a) Laden cell. (b) Uncharacterized cell type. (c) Partially depleted cell. Line = 10 μm.

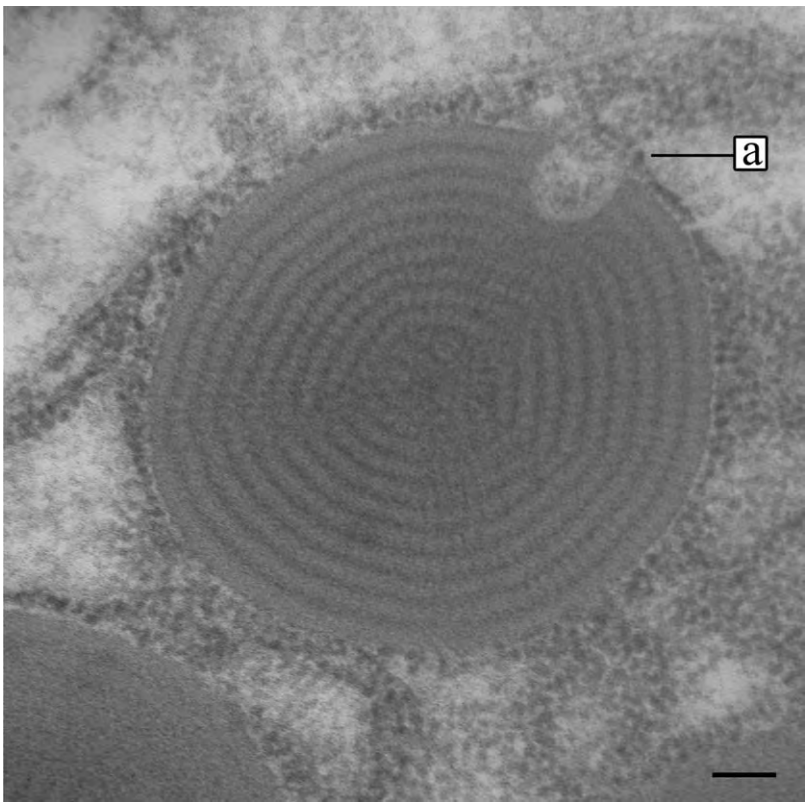

Fig. 10. *D. citri.* Cross section of an embedded PSG spherule from a laden cell in the block in Fig. 7. (a) Spherule is undergoing either storage or mobilization of secretory material in this area. Line = 0.1 μm.

because its basal cells were embedded in the squamous cells of the anterior PSG (Fig. 11g). The ASG therefore lifted directly out with the PSG during extirpations. The internal canal of the ASG was lined with cuticle from base to apex (Figs. 11i and 12b). This intima, in turn, was surrounded by a dense layer of lamellae radiating perpendicularly into the cytosol (Fig. 12c). Punctate material occurred at the base of each lamella (Fig. 12d). The junction of the ASG lumen and the PSG lumen (Figs. 11f and 13) underwent closure during processing and could only be given limited characterization. At least two apical membrane morphologies are evident—one morphology with a cuticular intima and lamellae radiating perpendicularly into the cytosol from it, and the other morphology without such features.

An enveloping sheath was not found in extirpated, plastic embedded PSGs of either species. In whole mounts of plastic embedded *B. cockerelli*, TEM revealed that a neighboring organ was invested by a narrow layer with numerous mitochondria that seemed to be part of the PSG where the two were tightly appressed (Fig. 14).

Discussion

Alimentary Canal. The midgut is looped among many Sternorryncha so that the esophagus–midgut transitional region is in contact with the midgut–hindgut transitional region (Fig. 1B). The conclusion of classical histologists that this "zone of contact" facilitates removal of excess water and nutrients ("filtration," Goodchild 1963, 1966) is still considered to be an accurate interpretation by insect physiologists (Chapman 1998). The configurations of the tubes in this zone, generally esophagus, midgut, malpighian tubules and hindgut are, taxonomically, very diverse, and the means by which contact is maintained (e.g., adherence, fusion, and ligation) is largely unknown. They may be chambered within the primitive epithelial walls of the midgut–hindgut transitional region, as in whiteflies (Cicero et al. 1995), within a sheath, or unchambered. We propose that when chambered, the inner complex of tubes is called the "filter organ," and the entire conglomerate structure (chamber walls + filter organ) is called the "filter chamber."

The makeup, whether cellular or noncellular, and origin, both organismic and phylogenetic, of the filter chamber sheath remains a mystery among Sternorryncha. Ponsen (1979, p. 19) addressed the wide diversity of sheathed and unsheathed filter organs sketched in the classical literature, but was not able to draw any conclusions in these regards. Because of the confusion in the literature as to the actual construction of filtering devices within various taxa, most need to be reexamined with modern techniques. Homology be-

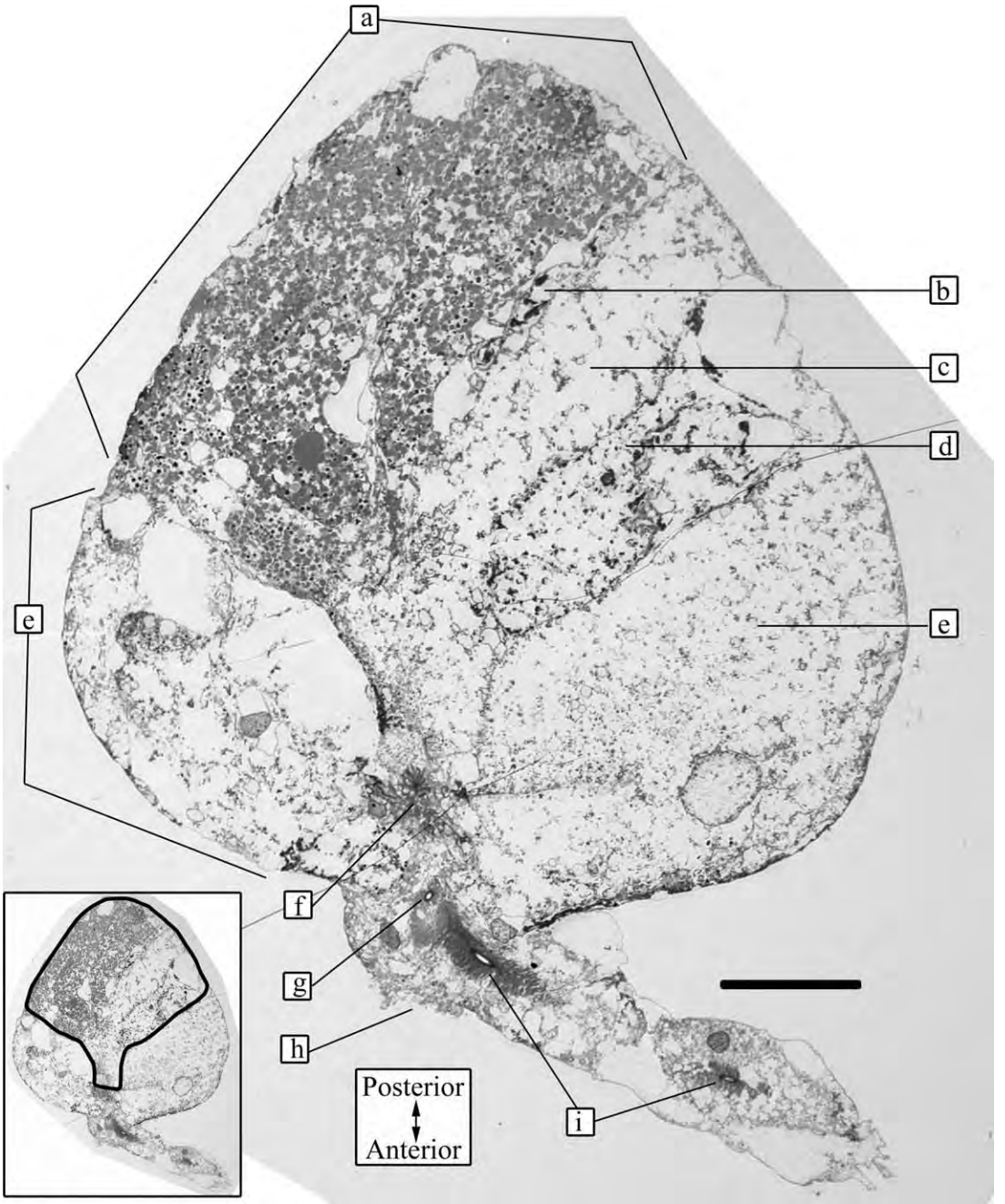

Fig. 11. Extirpated salivary glands of *D. citri*. The PSG is spherical and the ASG is digitate. Inset, demarcation of a funnel-shaped pattern of secretory cells. (a) Cells laden with secretory spheres. (b) Cells seem to show residue from depletion of secretory spheres. (c) Intervening cell type remains uncharacterized. (d) Continuation of depleted cells pointed to in b. (e) Lateral cells have no apparent secretory capacity. (f) Lumen of the PSG is closed, possibly because of hypertonic effects of extirpation. (g) PSG lumen seems to continue into the accessory gland. (h) Severed attachment site of the external salivary gland duct that extends to the oral complex. (i) ASG lumen continues to apex. Line = 30 μm.

tween the components of the filter chamber will best be determined with a basis in embryological organogenesis.

Earlier authors of the psyllid alimentary canal (see Introduction) indicated that the zone of contact is unchambered and may be twisted or helically wound. These classical dissections need to be rechecked for accuracy. As it stands now, the key familial characteristics of the psyllid alimentary canal can be tentatively established by combining what has been learned

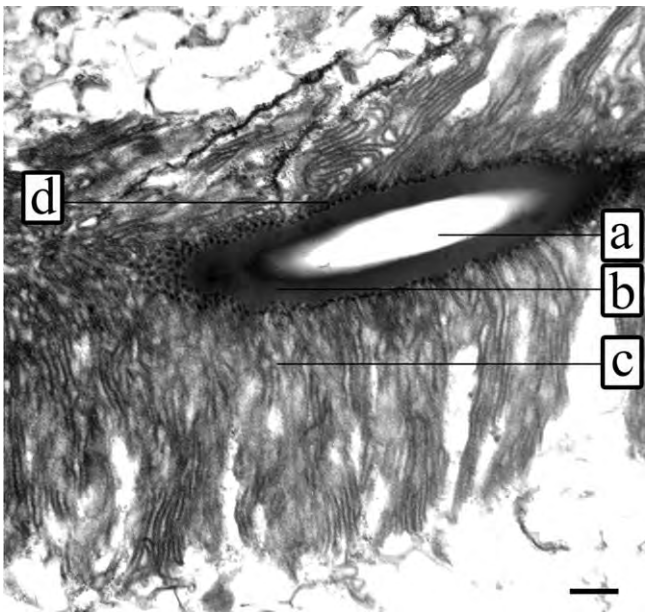

Fig. 12. Accessory gland lumen of *D. citri* pointed to in Fig. 11i. (a) Lumen. (b) Intima. (c) Lamellae, possibly endoplasmic reticula. (d) Punctate material occurs at the base of each lamella. Line = 0.5 μm.

here for *D. citri* and *B. cockerelli* with the classical works, and comparing them with other Sternorryncha. These key features include a relatively short midgut loop with four short, stout, well-spaced appendages, and a short, sheathed, tightly wound zone of contact with a trailing hindgut that is routed to the anus from the anterior end, next to the point of entry of the esophagus, rather than the posterior end (e.g., Snodgrass 1935, p. 384, fig. 209B). Also, the midgut may have a conspicuously swollen section immediately anal to the zone of contact (Fig. 1C, f and 1D, 2 Vent), before attenuating to a uniform girth along the rest of the loop (Fig. 1B and C, g).

Snodgrass' referral to V_3 as ventricular on entry, and hindgut on exit, seems valid because he indicates that malpighian tissue occurs just interior to the exit site (Fig. 1D, Mal). He draws this feature as an indefinitive ring of stubs, which probably represents omission of the long, tenticular shape, a common practice of that time (Wigglesworth 1956). However, in this study, malpighian tissue was not observed anywhere in *D. citri* or *B. cockerelli.* Although the points of entry and exit of conduits into and out of the filter chamber are conspicuous landmarks, there is no basis for using them as sites for the beginning and end of the organ types. The stretch of tube in antiparallel adherence to V_1 is provisionally referred to as the "inner hindgut" because it is the acceptor of filtrate from V_1. Studies on how to identify the beginning and end of the component alimentary organs are in progress.

Brittain (1923) and his followers labeled the four appendages coming from V_3 as malpighian, but they did not provide histological or physiological support to substantiate this. Substantiation is essential because a variety of tubular arrays, from simple to branched, and attachment sites from nodal to consecutive, have been referred to as diverticula (caecae) or malpighian tubules along the length of the midgut and the apical hindgut in the broader Hemiptera (Glasgow 1914, de Marzo and Marotta 2001). The tracheae attached to these appendages (Fig. 1E, q) are probably Brittain's "fine suspensory ligaments."

Marshall and Cheung (1974) developed models to explain how the cicada filter chamber extracts water from the ventriculus to the hindgut, but they did not consider the effects that the by-products of digestion might have on this process. It would seem that the effects could be significant because these byproducts move through to the end of the zone of contact while the extracted water is collaterally added to it. Both then move to the anus. Also, typically in insects, the malpighian tubules extract general blood-borne metabolic waste and route it to the hindgut. But because malpighian tubules have not been identified in the hemocoel of *D. citri* and *B. cockerelli*, disposal of blood-borne waste remains a mystery.

Study of well preserved specimens shows that upper V_1 is bulbous and inflated (Fig. 4f). We reason that this shape is a natural feature of the area, and contains the greatest amount of water fated for disposal. Points on lower V_1 further and further from the influx of food should have a consecutively decreasing need for extraction of water, and it is likely that the cytology changes correspondingly and gradually also. The involutions along the basal laminar interface between the inner hindgut and the bulb (Fig. 5b and c) do not compare well with the strong involutions seen in whiteflies (Cicero et al. 1995, p. 34, fig. 3), but the rest of the composite tube interface has yet to be characterized.

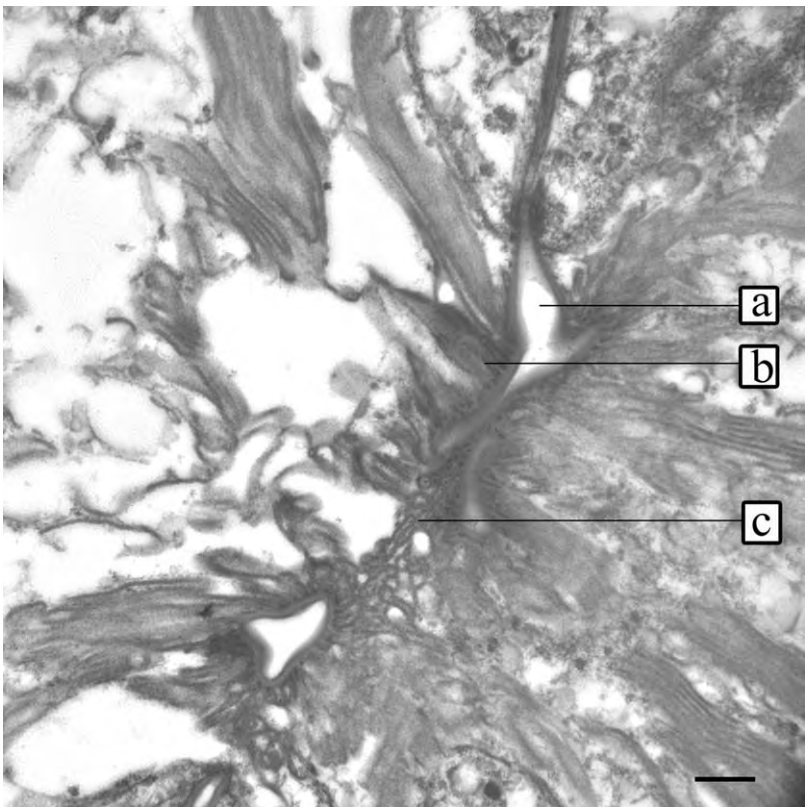

Fig. 13. *D. citri*. Close-up of the core PSG luminal complex pointed to in Fig. 11f. It is apparently a hub that is continuous with the accessory gland lumen and the salivary gland duct that leads to the mouth. At least two apical membrane morphologies occur in this area, but because of luminal closure, the associated cells and their positions in the luminal continuity could not be determined. (a) Intima lined lumen. (b) Lamellae, cf. Fig. 12c. (c) A second apical membrane morphology. Line = 0.5 μm.

These interpretations are based on the classical view that fluids are confined to the conduit system. However, the content and function of the interstitial space of the filter chamber surrounding the composite tube is not known. Therefore, the interstitial space could serve as a possible pool for holding excess water that also could pass directly into the inner hindgut. The opaque white color of V_2 and V_3 is interpreted to be due to the accumulation of stored food inside their lumina. The hyaline color of the filter chamber would, by that same reasoning, be due to its function as a hub for flow-through of materials involved in the filtration process.

Nothing is known of the origin of honeydew in psyllids. The extraction process could occur along the composite tube, or honeydew could be the residue from midgut digestion that reenters the chamber and mingles with adsorbed material from V_1 as it moves to the exit point, or both.

Salivary Glands. Drawings of the *P. mali* salivary gland (Saunders 1921) and the *P. buxi* and *T. urticae* salivary glands (Witlaczil 1885), show a spherical pattern of crescent-shaped cells arranged about a node which represents the authors' view of the lumen leading to the salivary duct. Saunders (1921, plate 3, fig. 22, and text) referred to the organ as the primary salivary gland, and noticed a secondary, cylindrical component emerging from the base, where the salivary duct attaches, but stopped short of defining it as the accessory salivary gland. For our purposes, this organ is called "accessory" in accordance with classical authors of other taxa who labeled it as such because of its eccentric, subordinate location and smaller size with respect to a larger PSG. Studies of the *D. citri* PSG (Xu et al. 1988) indicate an enveloping sheath to be present that harbors bacteria-like organisms. We have located this layer and identify it as belonging to a neighboring organ (Fig. 14).

It is generally accepted that the primary role of salivary glands is to produce secretory products for feeding, including the salivary sheath (Miles 1999). But unequivocal confirmation is needed to assert that the glands are the sole source of these products, and whether the PSG, ASG, or a combinatorial mix of secretions from both is involved in each salivary function in the various taxa concerned. Stylet sheaths are secreted by other hemipterans including aphids, cercopids, whiteflies (Valerio 1989, Freeman et al. 2001, Takemura et al. 2006), and certain psyllids (Brennan et al. 2001), but they have not yet been reported in the

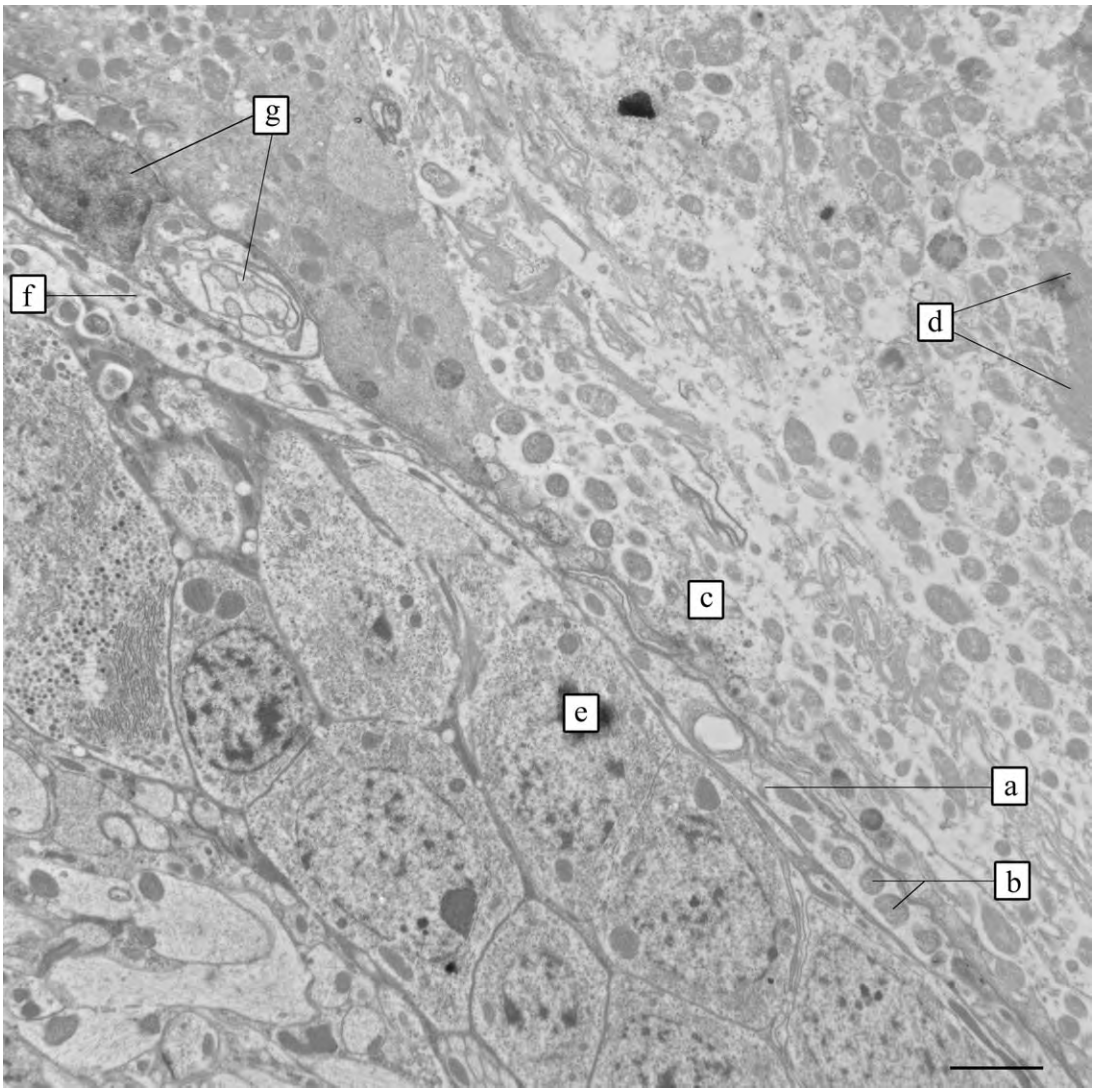

Fig. 14. Cross section of an area of the *B. cockerelli* prothorax showing the PSG and a tightly appressed neighboring organ. (a) Layer between PSG and neighboring organ. (b) Mitochondria. (c) PSG. (d) Brush border of the PSG lumen. (e) Neighboring organ. Layer in f continues around the adjacent organ, not the PSG. (g) Intervening material. Line = 2 μm.

two species featured herein. At least two cellular motifs were observed for the PSG of both psyllid species examined. Those rich with fully spherical spherules (Figs. 9a and 11a) are interpreted as *laden*, whereas those containing partially formed spherules (Figs. 9c and 11b and d) are interpreted as *depleted.* Both cell motifs were present in all specimens examined. A third cell type (Figs. 9b and 10c) remains uncharacterized.

In both the PSG and the ASG, cells likely need access to noncuticularized portions of the core lumen for their products to pass through the apical membrane into the ducts and then move to the mouth. Figure 11 provides some evidence for such an arrangement, based on the funnel-shaped configuration of secretory cells (Fig. 11, inset). Such wedge-shaped cells also comprise the accessory glands of *Bemisia tabaci* (Gennadius) (Ghanim et al. 2001, p. 29, fig. 6), conferring a "hesperidiform" shape to that organ. Indications are that only one common duct occurs in *D. citri* (Fig. 8d) rather than dual ducts, as found in *B. tabaci* (Harris et al. 1996a, p. 147). This study was not able to determine the point where the lumina of converging ducts coalesce so that salivary materials from left and right glands mix before they enter the single salivary channel of the stylet. The presence of one salivary channel in the stylets of these two species is consistent with the broader Sternorrhyncha (Forbes 1972, Ullman and McLean 1986, Harris et al. 1996a).

The function of the lateral cells of the PSG (Fig. 11e) cannot be surmised. The function of the entire ASG is a mystery because it is not separated from the PSG by a duct of its own, as occurs in *B. tabaci* (Harris

et al. 1996a,b); in addition, all of its cells seem isolated from the lumen by an intima (Figs. 11i and 12b). The basally punctate lamellae (Fig. 12 c and d) could be endoplasmic reticula. Their relationship to the metabolic processes of the ASG is currently under investigation.

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