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Histomorphological Characteristics of Digestive Organs of *Lutzomyia wellcomei* Fraiha, Shaw & Lainson (Diptera, Psychodidae)*

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Abstract. Sandflies are important vectors of tegumentary and visceral leishmaniasis in different countries. This study aimed at analyzing the histomorphological characteristics of digestive and reproductive organs in *Lutzomyia wellcomei* (Fraiha, Shaw & Lainson) using light microscopy techniques. Thirty females from the rural area of Nísia Floresta, Rio Grande do Norte state were selected, microsectioned and analyzed with an optical microscope using conventional hematoxylin-eosin staining. Results show three well-characterized regions in the digestive tube: the stomodeum, mesentery and proctodeum. The stomodeum is lined internally with a basal and epithelial membrane; the mesentery has a peritrophic matrix formed above the stomodeum lining consisting of the simple cubic epithelium overlapping the conjunctive, also common to a capsule of gonadal lining; and the proctodeum, which structurally resembles the mesentery, but with a pyloric sphincter limiting the posterior midgut. Enveloping the digestive tube is the peritoneal membrane, of mesodermal origin. Richly-detailed internal morphological characteristics of *L. wellcomei* have widened knowledge of this *Leishmania braziliensis* (Vianna)-transmitting species, an etiologic agent of American tegumentary leishmaniasis.

Keywords: Histomorphology; Morphology; Phlebotomine; Tegumentary Leishmaniasis; Vector.

Características Histomorfológicas de Órgãos da Digestão de *Lutzomyia wellcomei* Fraiha, Shaw & Lainson (Diptera, Psychodidae)

Resumo. Os flebotomíneos se destacam como importantes transmissores das leishmanioses tegumentar e visceral em diferentes países. O objetivo desse estudo foi analisar características histomorfológicas de órgãos da digestão de *Lutzomyia wellcomei* (Fraiha, Shaw & Lainson) por meio de técnicas de microscopia. Trinta fêmeas provenientes de área rural do município de Nísia Floresta, Rio Grande do Norte foram selecionadas, microseccionadas e analisadas por microscopia ótica. Métodos de coloração histológica por hematoxilina-eosina foram empregados nas análises. Os resultados mostram o trato digestório com as três regiões bem caracterizadas, estomodeo, mesêntero e proctodeo. O estomodeo possui uma membrana basal e uma membrana epitelial que o reveste internamente; o mesêntero possui matriz peritrófica formada acima do revestimento do estomodeo constituído de epitélio cúbico simples sobreposto ao conjuntivo comum também a uma cápsula de revestimento gonadal; e o proctodeo que se assemelha estruturalmente com o mesêntero, possuindo ainda, uma região pilórica limitando o intestino médio do posterior. Envolvendo o tubo digestório, está a membrana peritonial, de origem mesodérmica. As características morfológicas internas, observadas com riqueza de detalhes em *L. wellcomei* ampliam o conhecimento acerca dessa espécie, transmissora de *Leishmania braziliensis* (Vianna), agente etiológico de leishmaniose tegumentar americana.

Palavras-Chave: Flebotomíneo; Histomorfologia; Leishmaniose Tegumentar Americana; Morfologia; Vetor.

Tegumentary leishmaniasis (TL) is frequently caused by *Leishmania major* Yakimoff & Schokhor and *Leishmania tropica* Wright in the Old World, and by *Leishmania braziliensis* Vianna and *Leishmania mexicana* Biagi in the New World. Ninety percent of TL cases occur in Afghanistan, Iran, Saudi Arabia, Syria, Colombia, Peru, and Bolivia (Deusjeux 2004; Reithinger *et al.* 2007). In all countries where it is found, TL has been shown to be a public health problem, since it can cause destructive, disfiguring and incapacitating lesions with important psychological and social impacts on those affected. The tegumentary form is described in four of the five regions of Brazil, with highest incidences in the North and Northeast (Ministério da Saúde 2010).

Parasites are transmitted by the bite of different sandfly species. They are small, holometabolous insects measuring approximately three millimeters in length, with very pilose bodies. Females need to ingest blood in order to develop their ovaries and reproduce (Killick-Kendrick & Killick-Kendrick 1987).

Sandfly species include *Lutzomyia wellcomei* Fraiha, Shaw & Lainson (Fraiha *et al.* 2004), insect vector of *Leishmania braziliensis* (Vianna), an etiologic agent of American tegumentary leishmaniasis. *L. wellcomei* was initially described only in the Amazon Region (Lainson *et al.* 1973). It was recently found for the first time in Rio Grande do Norte (Cortez 2006). In this state tegumentary leishmaniasis is caused by *L braziliensis* and occurs mainly in the highland region of Alto Apodi (Oliveira *et al.* 2004).

The transmission mechanism of the parasite depends on interactions between the parasite and vector in the sandfly's digestive tract. When females bite they ingest a blood meal and may acquire or transmit species of *Leishmania*. Once in the digestive tract of an invertebrate, the protozoan assumes its promastigote form, differentiating into a procyclic promastigote,

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avoiding the expulsion of the parasite from the midgut of the vector. Sequentially, the parasite assumes its infectious metacyclic promastigote form, a stage in which it migrates to the oral parts of the insect, allowing it to infect other hosts during its next meal (Sacks 1989).

Studies related to morphology may contribute to elucidating questions concerning the development and transmission of etiologic agents. The organic microstructure and internal tissue of sandflies is little studied, likely because of its small size. The use of histological procedures in the study of sandflies, as well as microscopic techniques, may produce good results, provided that adequate procedures are followed when preparing organs and tissues.

Most studies published on this topic were conducted decades ago, predominantly involving Old World species (Lewis & Minter 1960).

Recent published work includes research on the parasite-vector interaction or formation of the peritrophic membrane and blood digestion (Blackburn *et al.* 1988; Walters *et al.* 1995).

Guzman *et al* (1994) and Abassy *et al* (1995a) used histological methods to study food physiology and embryonic development in Old World species *Phlebotomus dubosqi* Neveu-Lemaire and *Phlebotomus papatasi* Scopoli, under a light microscope. The authors obtained satisfactory results, indicating that these methods are practical tools in studies on sandfly morphology and physiology (Guzman *et al.* 1994; Abbassy *et al.* 1995a, 1995b, 1995c).

Thus, the present study aimed at analyzing the histomorphological characteristics of digestive organs in adult female *L. wellcomei* in order to contribute to a better understanding of their biological cycle, as well as widening knowledge of this species, recently described in Rio Grande do Norte.

MATERIAL AND METHODS

Sandfly capture. Thirteen collections were made on random days between September 2009 and September 2010. Insects were captured by aspiration with a Castro aspirator used on the walls of a brick tank housing an armadillo (*Euphractus sexcintus* Linnaeus). Next, they were placed in nylon cages, which were transported in plastic bags containing moist cotton pads to maintain humidity. Insects were taken to the Laboratory of Entomology, Department of Microbiology and Parasitology, Bioscience Center of the Federal University of Rio Grande do Norte.

Sandfly identification. Sandflies captured were dissected with the help of entomological tweezers, under a ZEISS Stemi DV4 stereomicroscope. Dissected parts that were used only for species identification were removed and immediately immersed in 70% alcohol inside cone-shaped plastic tubes. Species identification was based on dissected heads, which were individualized in plastic tubes with numbers corresponding to slides containing the abdomens. Sandflies were identified according to the classification proposed by Young & Duncan (1994), after being clarified and mounted between the slide and cover slip.

Histological processing. A total of 30 specimens of *L. wellcomei* were fixed in 5% formaldehyde for 12 hours. Sandflies were then submitted to routine histological techniques and microsectioned at a thickness of 5µm for the manufacture of histological slides. Microsections of longitudinal cuts of the abdomen, digestive tube and gonads underwent conventional Harris hematoxylin and eosin (HE) staining protocols. The resulting slides were microphotographed with a Nikon DXM 1200 digital camera coupled to an Olympus BX41 microscope, for comparative analyses.

RESULTS AND DISCUSSION

General histomorphological aspects of the digestive tube of adult *L. wellcomei* were observed for the first time by means of an optical microscope, allowing analysis of the insect's morphological traits.

The optical microscope shows that the digestive tube of female *L. wellcomei* (Figure 1) is composed of a tube of epithelial cells divided into three regions, based on embryonic origins and physiological functions of the foregut, midgut and hindgut as in other groups of insects.

The first is the foregut or stomodeum (Figure 1a), the most anterior portion of the tube, consisting of the mouth, oral cavity (where the salivary glands emerge), pharynx, esophagus, diverticulum and proventriculum. The diversity of oral parts mirrors the structural diversity of the digestive tract, with an enormous degree of specialization, varying with the specific type of diet (Klowden 2002).

The pharynx, which is related to food ingestion, has well developed muscles (Vale *et al* 2012). The esophagus, generally tube-shaped, connects the pharynx to the diverticulum (Terra & Ferreira 1994; Chapman 1998). Internally, the walls are lined with a membrane called intima, which is the continuation of the external cuticle of the tegument, containing hair, spicules or teeth; next is a fine epithelial layer, which is the continuation of the epidermis of the body wall and secretes the intima. Enveloping the epithelial is the basal membrane composed of a thin layer of cells, as described by Buzzi (2002).

The second region is called the mid-thoracic intestine, stomach or mesentery (Figure 1b; Figure 2), where a large part of chemical digestion occurs. It is lined with cubic cells, likely of the secretory and stem variety, the latter also known as mother cells. Food digestion in the foregut occurs by means of salivary glands, while enzyme production takes place in the midgut through the epithelial lining and formation of a peritrophic matrix (Figure 3a). Its function is to serve as a physical structure to protect the intestinal epithelium from food content, as well as pathogenic microorganisms present in ingested blood (Richards & Richards 1977). Proteolytic enzymes penetrate this matrix to initiate blood digestion, and any laggard *Leishmania* protozoa occurring on the periphery will be eliminated (Peters 1992; Terra & Ferreira 1994; Oliveira *et al.* 2005). When hematophagia takes place (Figure 3b, Figure 4a), intestinal contents are separated into two compartments: the endoperitrophic space and ectoperitrophic space (Terra & Ferreira 1994). The stomach is lined with a simple cubic epithelium consisting of secretory cells overlapping the conjunctive, which is also part of the gonadal lining (Figure 3c);

Blood accumulation provokes a distension of the stomach wall (in purple) (Figure 5), likely owing to the presence of proteins and proteoglycans from the peritrophic matrix; at this site food remains until it is digested. In mosquitoes, the volume of blood ingested varies according to the mosquito: *Anopheles* ingests 0.002mL and *Culex* 0.003 mL (Lehane 2005). In *Aedes aegypti* Linnaeus mosquitoes the matrix does not form when sugared substances pass through the stomach (Consoli & OLIVEIRA 1994). In *Lutzomyia longipalpis* Lutz & Neiva the peritrophic matrix starts to form about 1 hour after blood ingestion, and after 24 h a thin structure can be seen separating the blood from intestinal cells. This matrix is chitinous and composed of a fibrilar and granular layer, the latter in contact with the food (Secundino *et al.* 2005).

After parasites are ingested by sandflies along with blood, they position themselves at the center of the food content and multiply profusely in the first 48 hours. This is when the digestive process occurs and the peritrophic matrix forms around the food

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Figure 1. Longitudinal section of the digestive tract of.*Lutzomyia wellcomei*: a- foregut, b- stomach, c – hindgut (scale – 200µm - HE).

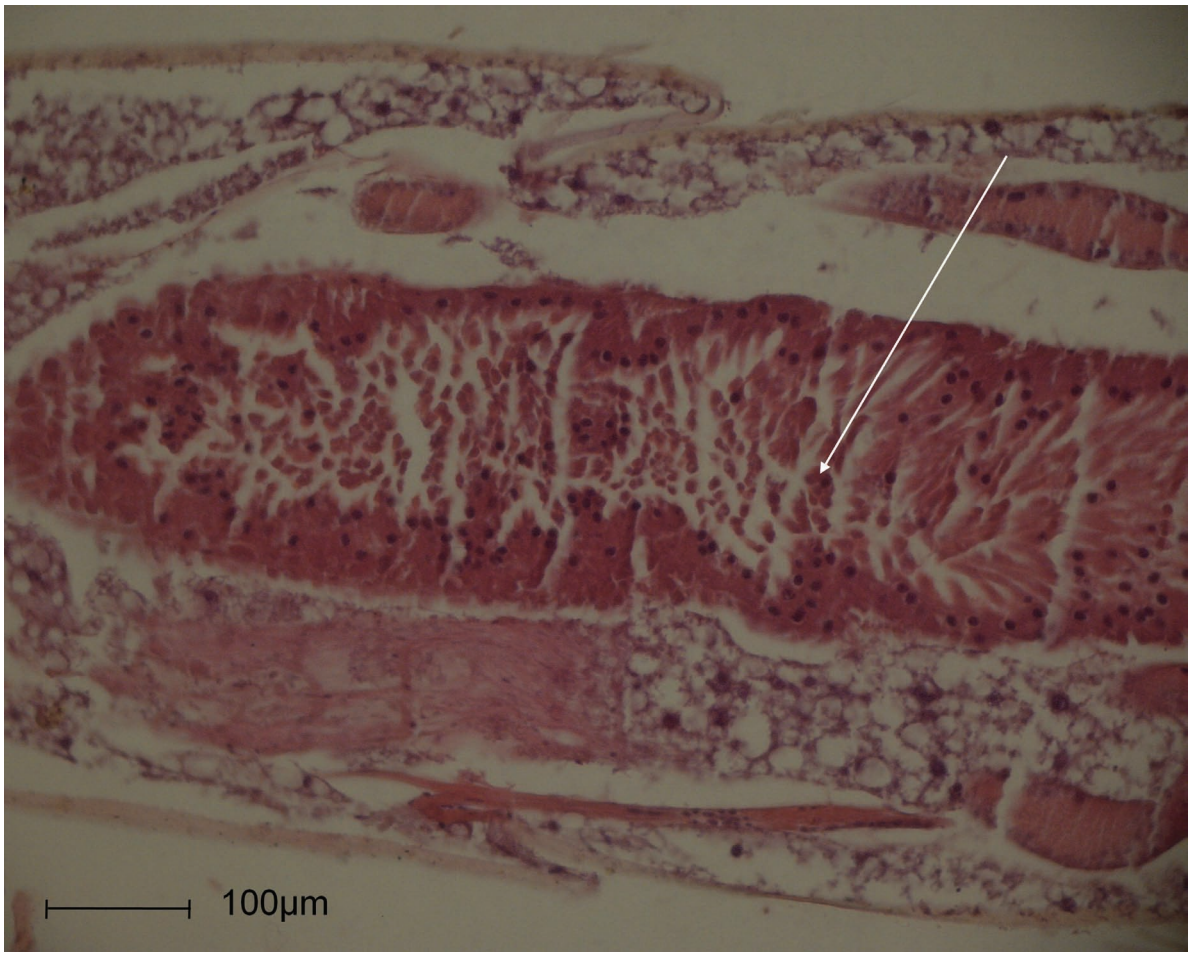

Figure 2. Longitudinal section of the stomach region of *Lutzomyia wellcomei*. The arrow indicates the lumen (scale – 100 µm - HE).

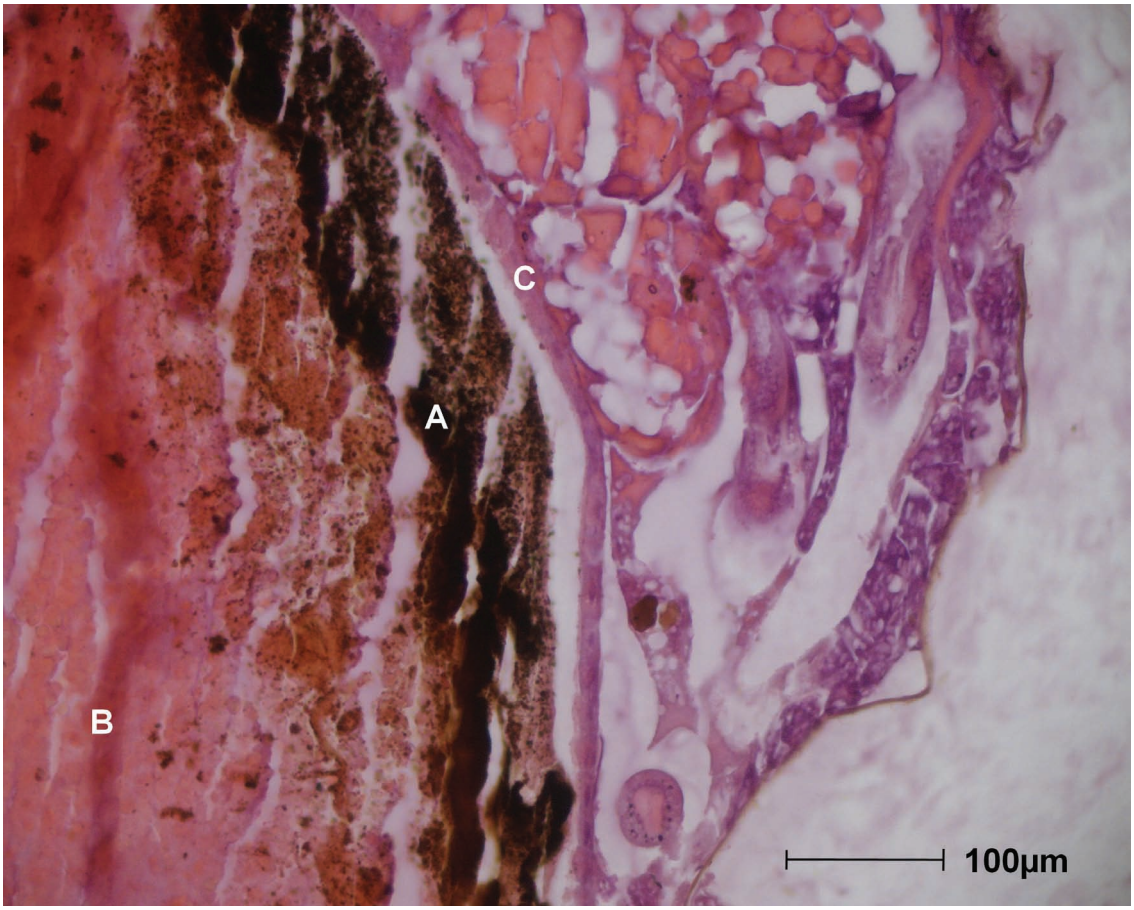

Figure 3. Longitudinal section: a – peritrophic matrix, b- digested blood, c- gonad lining (scale - 100 µm - HE).

Figure 4. Longitudinal section – photomicrograph of the entire abdominal region of *Lutzomyia wellcomei* – a- blood meal stored in the stomach; b - gonad (scale – 200µm – HE).

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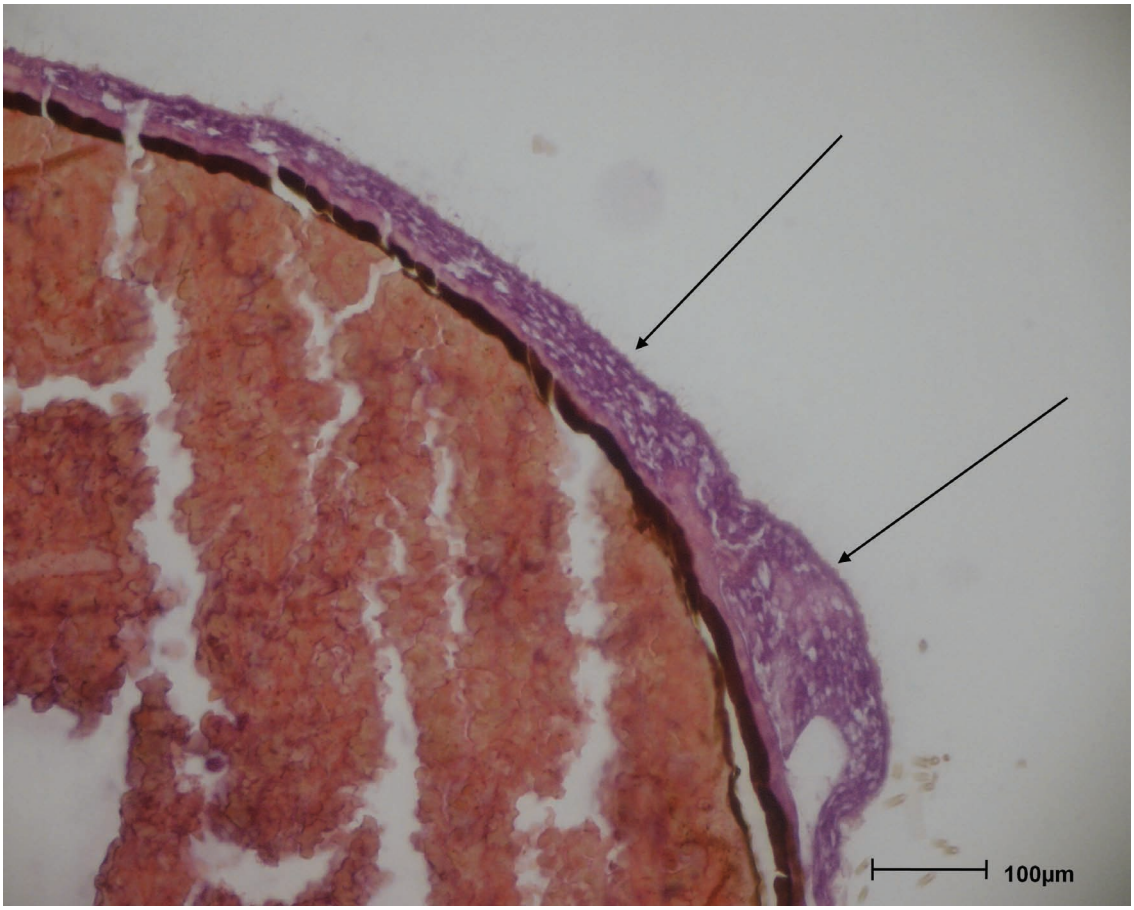

Figure 5. Longitudinal section: distension of the stomach wall. Arrows indicate stomach wall distension (in purple, scale 100 µm - HE).

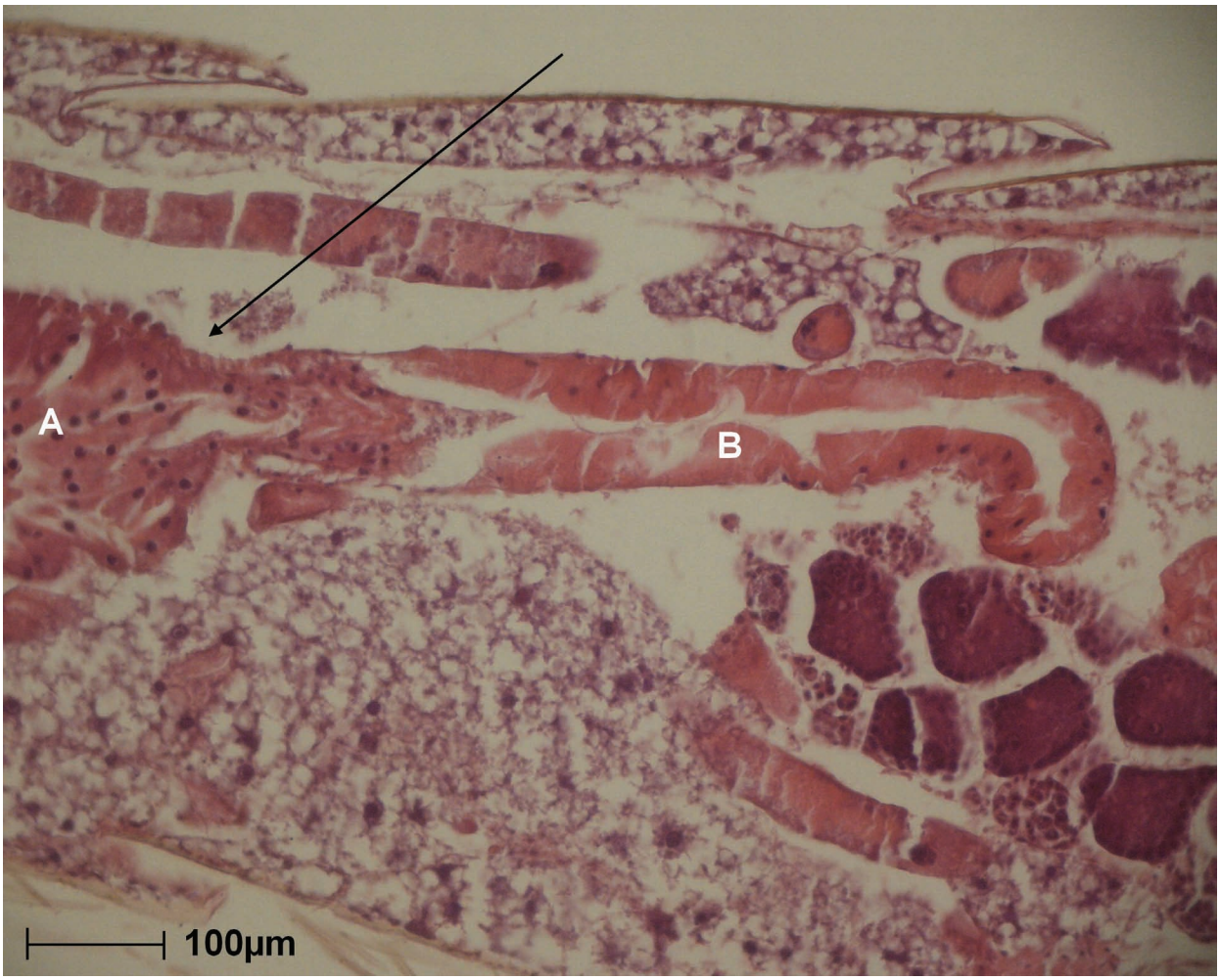

Figure 6. Longitudinal section: a- the most distal region of the stomach, b- hindgut, the arrow indicates the pyloric region (scale – 200µm – HE).

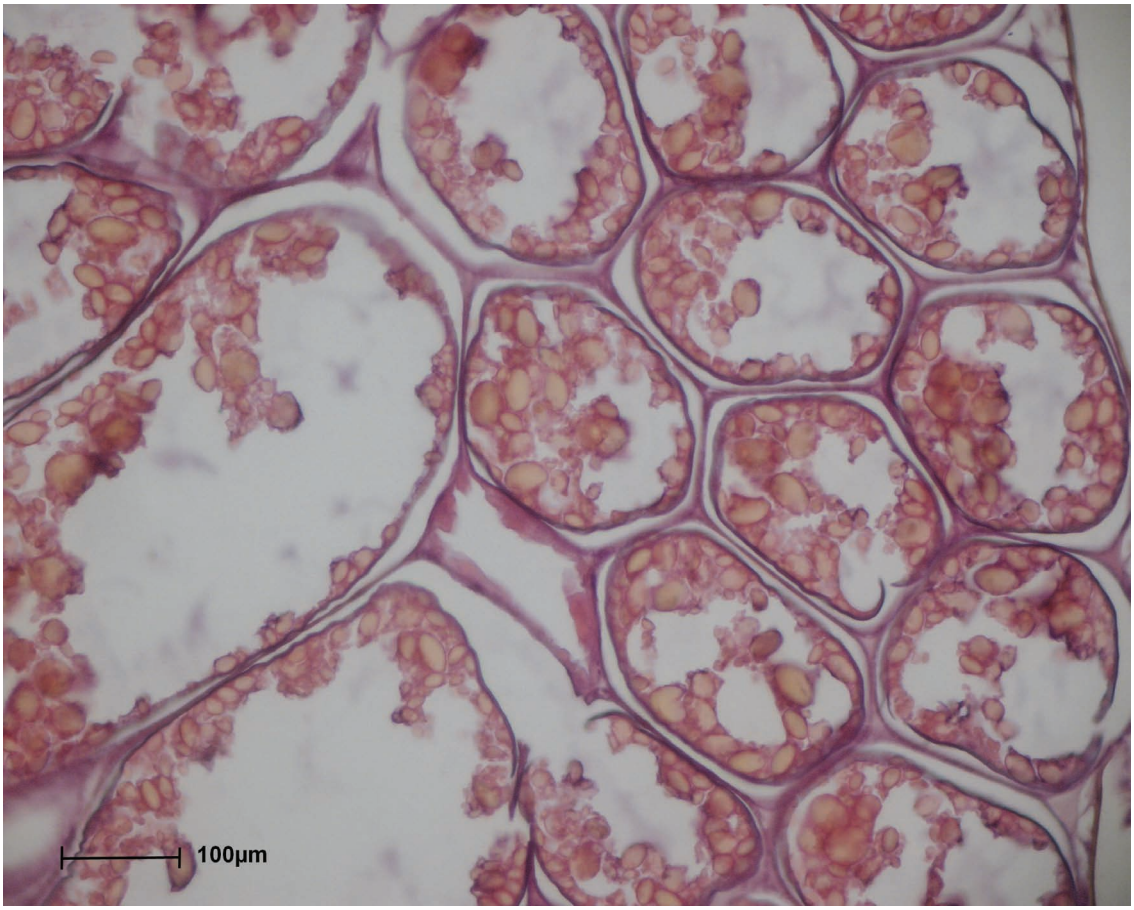

Figure 7. Longitudinal section: Ovary of *Lutzomyia wellcomei* containing eggs (scale – 100 µm - HE).

item. After 2 to 5 days, this membrane and the food processed start to be excreted, passing through the pylorus and the ileum. Depending on the species, after five days or more, egg development is complete and oviposition initiates (Lawyer *et al* 1990; Sacks 1989).

In contrast to mosquitoes, *L. wellcomei* may not have pyloric ceca in the stomach or hindgut, since they not anatomically or histologically visualized. Nor were these structures visualized in mature *L. longipalpis* larvae (Vale *et al.* 2012).

The third region of the digestive tube is called the hindgut or proctodeum (Figure 1c, Figure 6b). It has an absorptive function and pyloric sphincter (Figure 6b), limiting the posterior midgut, it starts where Malpighi tubes are inserted Sherlock *et al.* (1988). This portion of the intestine resembles the midgut, though with a smaller diameter. However, Buzzi reports that in insects it is identical to the foregut, the only differences being smaller epithelial cells and the presence of a thin flexible waterproof cuticle Buzzi 2002). In the present study, we did not diagnose this cuticle in sandflies. In *L. longipalpis* specimens, larvae have a short hind intestine and slender ileum.

Internally, the midgut and hindgut have no cuticle lining. The entire digestive tract is straight, with a small diameter and short in relation to the body length, identifying the hematophogous (blood-feeding) habit of *L. wellcomei*. This demonstrates a similarity with other insects that feed on hard tissues from plants and other animals, since the digestive tract is almost always shorter than body length. The region outside the digestive tract near the hindgut is where the gonads (Figure 3c, Figure 4b, Figure 7) are situated, consisting of several chambers containing the eggs. This characteristic gonad structure was observed in mature specimens. Gonadal morphology of different stages of sexual maturation was not the objective of the present study, although it may be considered as a perspective for future research.

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