

ULTRA MORPHOLOGY OF THE DIGESTIVE SYSTEM OF *Anastrepha fraterculus* AND *Ceratitis capitata* (DIPTERA TEPHRITIDAE)

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ABSTRACT

Anastrepha fraterculus and *Ceratitis capitata* are widely distributed fruit flies that cause significant damage to fruit crops in tropical and temperate regions. The economic importance of these flies has resulted in numerous studies of their biology, with particular emphasis on their control and management. However, various aspects of the biology of these species are still poorly understood. In this work, we used scanning electron microscopy (SEM) to examine the external anatomy and organization of the digestive system in these two species. Adult males and females of *A. fraterculus* and females of *C. capitata* were dissected in physiological saline solution, and the digestive tracts were removed and prepared for microscopy. SEM showed that the crop was covered by a strong muscular layer that consisted of circular fibers connected by longitudinal fibers; this arrangement was probably related to the post-feeding behavior of these flies in which the crop contents are regurgitated and reingested. The size of the rectum varied and was probably related to the different body sizes of the two species.

Key words: Gut anatomy, scanning electron microscopy, Tephritidae

INTRODUCTION

The digestive tract of insects is a continuous tube that extends from the mouth to the anus and passes through different regions containing valves and sphincters [4,10,22]. Morphologically, the insect digestive tract is divided into foregut (stomodeum), midgut (mesenteron, stomach) and hindgut (proctodeum), with the foregut and hindgut being of ectodermal origin, and the midgut being of endodermal origin [10].

The three regions of the digestive tract are very highly specialized: the foregut is involved in the intake and softening of food and/or the beginning of digestion, the ventricle or midgut is involved in the digestion and/or absorption of nutrients, in addition to producing the peritrophic membrane, and the hindgut is involved primarily in the absorption of nutrients and reabsorption of water and essential ions

that would otherwise be discharged along with the feces [4,6,9-11,22].

One of the first investigations of the anatomy of the digestive tract in adults of the family Tephritidae (Diptera) was that of Petri [20], who described the importance of the digestive tract of *Dacus oleae* in the release of symbiont bacteria close to the eggs at the moment of oviposition. Although this article described very little of the anatomy of the digestive tract, the tubular shape of the rectum was nevertheless very clearly illustrated.

The digestive tract of adult tephritids has a basic structure that consists of the gullet and its structures (esophageal diverticulum, cyst and part of the esophageal valve) (all of which form the foregut), the ventricle, i.e., the gullet itself, which consists of a simple, long tube that ends in the pyloric valve where the Malpighian tubules are connected, followed by the ileum and the rectum, which contains the rectal valve, sinus and rectal glands [12]. The digestive tract of adult *Anastrepha fraterculus* has a short gullet with an esophageal diverticulum followed by a short

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duct that ramifies to form the crop duct and a wide, bladder-like crop. After this ramification, there is a round cardiac valve (foregut or stomodeum), a long stomach with convolutions in the abdominal region (midgut or mesenteron), and finally the ileum and rectum, the walls of which contain the rectal glands (hindgut or proctodeum).

Many tephritid species, commonly known as true fruit flies, are agricultural pests of great economic importance. The females lay their eggs on fresh plant tissues, where the larvae develop. *Anastrepha* is a neotropical genus, with many species that infest commercial fruits. *Anastrepha fraterculus* is a generalist species that is very abundant in orchards [21]. *Ceratitis capitata*, the Mediterranean fruit fly (or medfly), has a worldwide distribution and infests a large number of fruit species. Together, *A. fraterculus* and *C. capitata* are very important fruit pests that infest more than 400 fruit species and cause considerable economic losses by reducing the market value of fresh fruit by destroying the fruit pulp or by staining the fruit [15].

The economic importance of these pests has resulted in many studies of these flies, with particular emphasis on their control and management [1,13]. Nevertheless, various basic aspects of their biology remain poorly understood. In this work, we used scanning electron microscopy (SEM) to examine the internal anatomy of *A. fraterculus* and *C. capitata*, particularly the digestive system. Descriptive studies such as this one may provide insights on aspects such as the relationship between these flies and their intestinal symbiotic bacteria that could be potentially useful in developing control programs for these pests.

MATERIAL AND METHODS

The flies were reared from natural infested guava fruits (*Psidium guayava*) collected in Jundiá (SP). The digestive tracts of adult males and females of *A. fraterculus* (Wiedemann) and females of *C. capitata* (Wiedemann) were removed in insect physiological saline using a stereomicroscope, fixed in Bouin solution for 24 h and then transferred to 70% ethanol. The tissues were subsequently dehydrated in an acetone series (70-100%), dried in a Balzers critical point drying apparatus and mounted on aluminum holders prior to coating with carbon and gold in a Balzers Sputtering apparatus. All preparations were analyzed and photographed using a Jeol P-15 scanning electron microscope and Neopan 200 film.

RESULTS

The following descriptions and figures are based on *A. fraterculus* and, unless otherwise indicated, there were no significant differences in the corresponding structures of *C. capitata*.

The crop duct was a long, narrow tube that ended in the crop, a wide, bladder-like structure covered by a strong muscular layer; the crop duct was innervated by the stomodeal nerve (Figs. 1A,B and 2A). Longitudinal muscles connected the circular muscle fibers (Figs. 2B and 3B).

The cardiac valve, which produced the peritrophic membrane, was round but flattened in the antero-posterior direction, and had very conspicuous longitudinal grooves on its surface. Between the cardiac valve and the midgut there was a short, narrow connection that corresponded to the neck of the proventriculus seen in other insects (Figs. 3B and 4A).

In the thoracic region, this narrow connection was followed by a straight midgut with nodules on

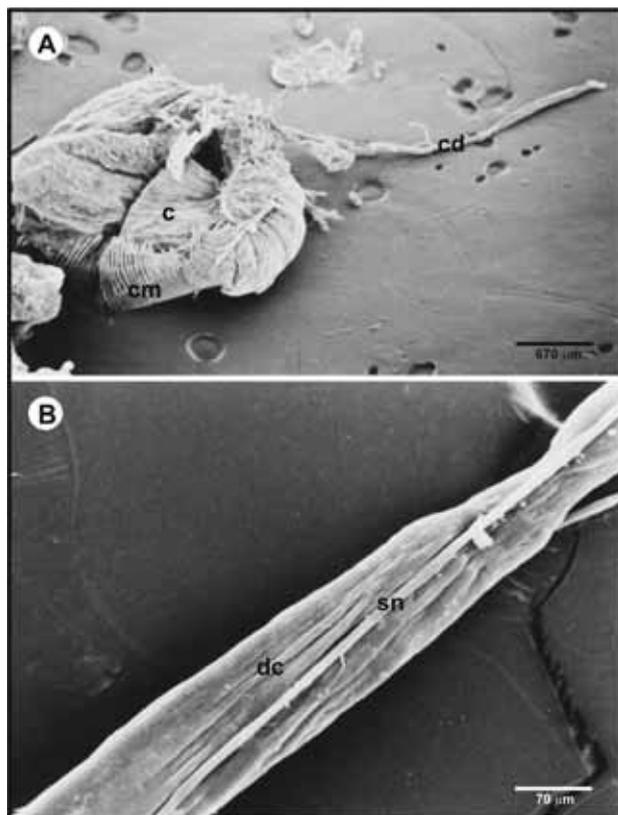


Figure 1. A. General view of the crop (c) and duct connecting the cardiac valve (cv) to the esophagus. Note the strong muscle layer covering the crop. B. Detail of the crop duct (dc) in a male of *A. fraterculus*. cm - circular muscle, sn - stomogastric nerve.

its dorsal portion (Fig. 3B). The midgut contained longitudinal and circular muscle fibers, although the former were located more peripherally and were more abundant on the side and base of this part of the ventricle (Figs. 4B and 5A). The nodules were apparently formed by the action of the circular muscle fibers (Fig. 5A). This region received numerous tracheal branches with subdivisions that developed among the muscle fibers and penetrated the epithelium (Fig. 5A).

The abdominal region of the ventricle showed slight undulations in the central portion and had muscle fibers distributed regularly over its surface (Fig. 5B). In contrast, the distal region was very rough and the distribution of muscle fibers was very unclear. Since the muscle fibers were not very evident, it was impossible to distinguish the longitudinal fibers. There were numerous branches

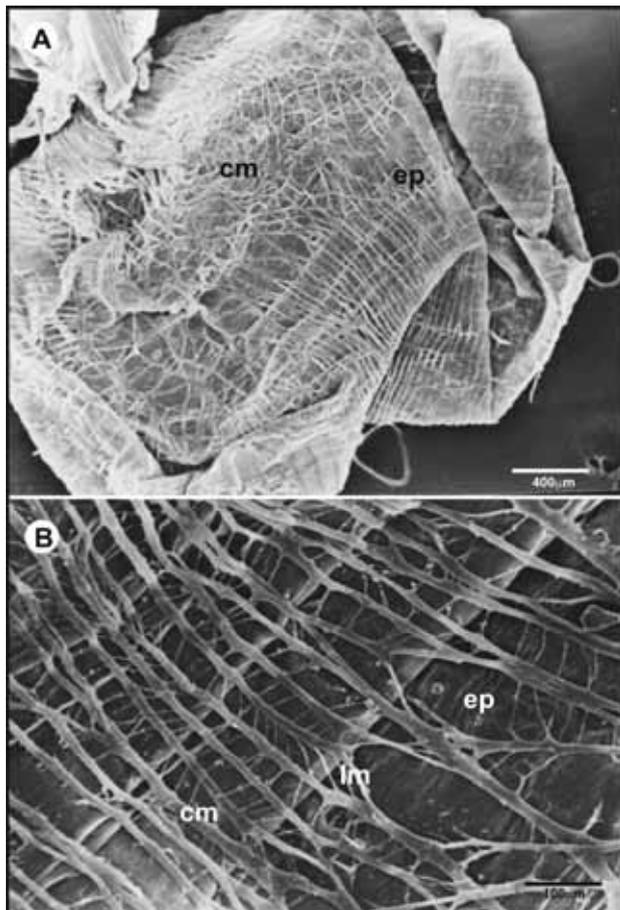


Figure 2. A. The crop of a female *A. fraterculus* showing the large layer of circular muscle (**cm**). B. Detail of (A) showing the longitudinal muscle layer (**lm**) connecting the circular muscle fibers. Note the epithelium (**ep**) beneath this muscle.

of the tracheal system over the entire surface of the ventricle, with ramifications that penetrated between the muscle fibers, as in the anterior region (Figs. 5B and 6A,B). The transition from the stomach to the ileum was sudden and involved a change in the arrangement of the circular muscle fibers, which in the ileum were more organized (Fig. 7B), and the insertion of Malpighian tubules.

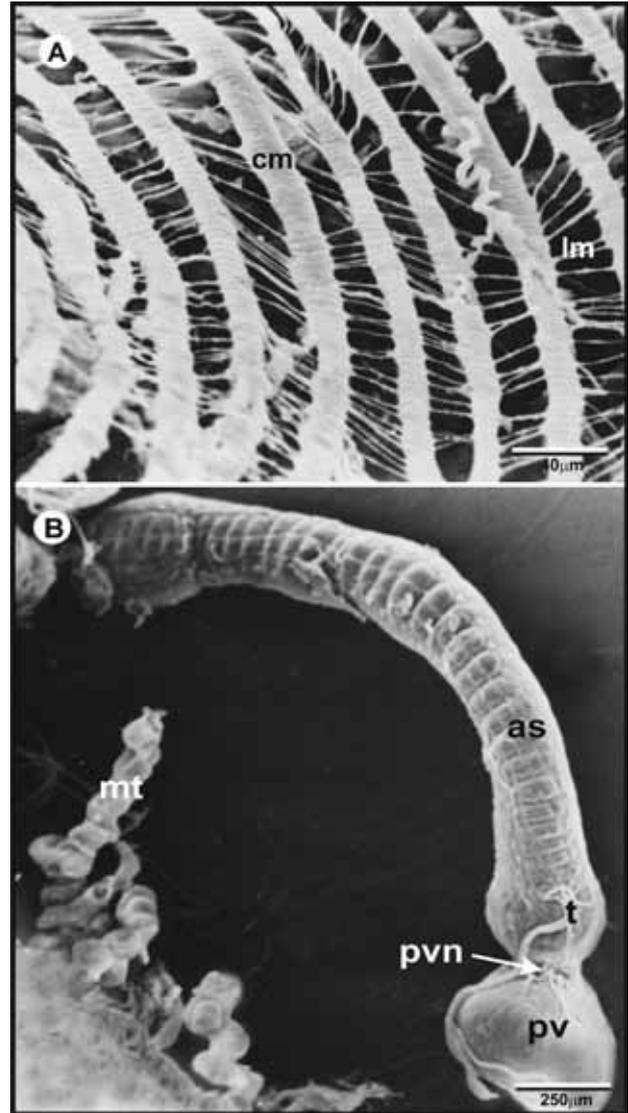


Figure 3. A. Detail of the crop muscle layer in a male *A. fraterculus* showing its similarities with that of females. B. General view of the proventriculus (**pv**) and midgut (**as**) of a female showing the round proventriculus with its tracheal branches (**t**), the narrow connection between the proventriculus and the midgut (neck of the proventriculus - **pvn**) and the undulations on the anterior dorsal surface of the midgut. **cm** - circular muscle layer, **lm** - longitudinal muscle layer, **mt** - Malpighian tubules.

The ileum had a basic organization that consisted of a very well developed circular muscle layer and a sphincter at the entrance to the rectum (Figs. 7A,B, and 8A,B). The rectum in *A. fraterculus* was much larger than in *C. capitata* but had a less developed layer of circular muscle (Figs. 7A and 9A). The anterior region of the rectum contained four rectal pads in a two-by-two arrangement that ran in opposite directions (Fig. 9A,B). In *A. fraterculus*, the rectal pads were 1.5 fold larger than in *C. capitata* (Fig. 10A,B), however

those of *C. capitata* had longitudinal striae that were more pronounced than in *A. fraterculus*. The rectal pads of *A. fraterculus* and *C. capitata* were supplied directly by the trachea, which penetrated the pads in a morphologically distinct region (Fig. 9A).

The rectum communicated with the external environment via a rectal-anal connection. This region consisted of an anterior portion with strong circular muscle and a posterior portion that was thinner and had no conspicuous muscle layer. This latter portion was connected to the cloaca.

DISCUSSION

Circular and longitudinal muscle fibers cover the crops of most insects, including ants [4,5,7,9], with

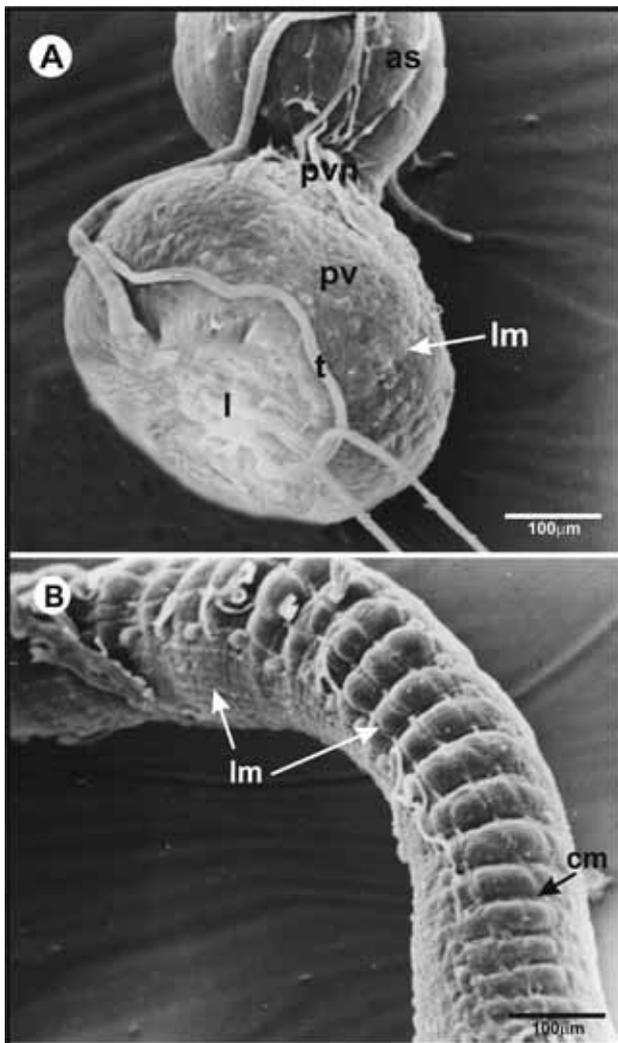


Figure 4. A and B. Details of Fig. 3A and B. In A, note the slight depression caused by the longitudinal muscle layer. B. Anterior midgut (as) with scattered longitudinal muscle fibers and the slight depression caused by circular muscle. Note that there are more longitudinal muscle fibers on the lateral surface than on the dorsal surface. cm - circular muscle layer, l - lumen, lm - longitudinal muscle, pv - proventriculus, pvn - neck of proventriculus, t - tracheal branch.

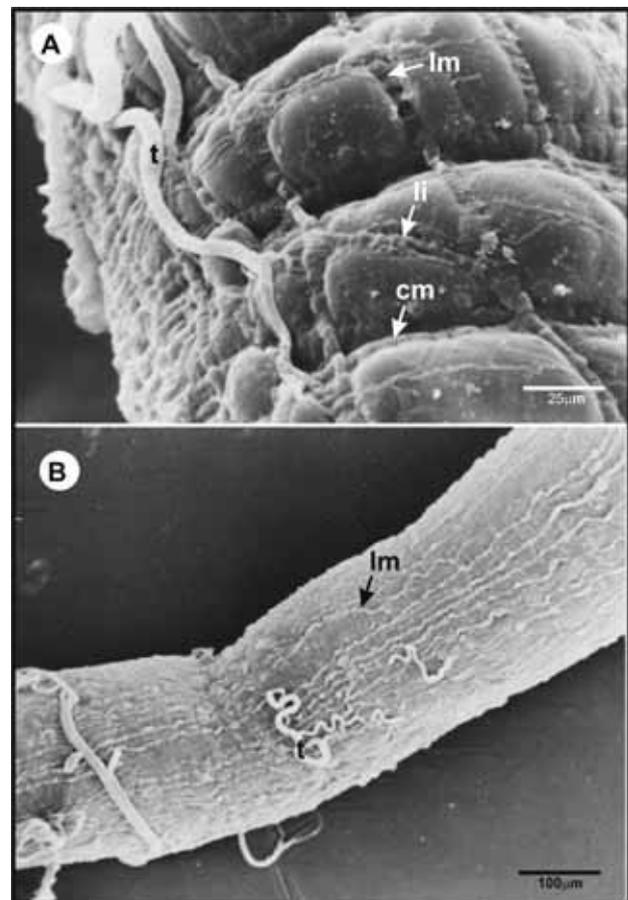


Figure 5. A. Detail of the anterior midgut wall of a female *A. fraterculus* showing the network formed by the circular and longitudinal muscle fibers. Note that the longitudinal muscle fibers produce bridge-like connections between each other. The circular fibers produce some “rings” deep in the midgut wall. B. General view of the middle portion of the midgut in a male of *A. fraterculus*. cm - circular muscle layer, l - lumen, lm - longitudinal muscle layer, t - tracheal branch.

the circular muscle layer generally being external to the longitudinal layer [22]. The presence of a layer of strong circular muscle around the crop may be important for post-feeding bubbling, to obtain more dehydrated food. After feeding on liquid food, the flies regurgitate their crop contents in small droplets and then re-ingest them, a behavior that may serve to eliminate excess water in the diet [14]. Muscle fibers have an important role in this regurgitation and in the transportation of food to the midgut.

Our findings for *A. fraterculus* and *C. capitata* differed from those for *Bactrocera dorsalis* in which there is a layer of strong, circular muscle but no longitudinal muscle around the crop [17]. However, the shape of the cardiac valve was similar to that of *B. dorsalis* [17], with the rough outer surface of this valve being attributable to circular muscle fibers and to the

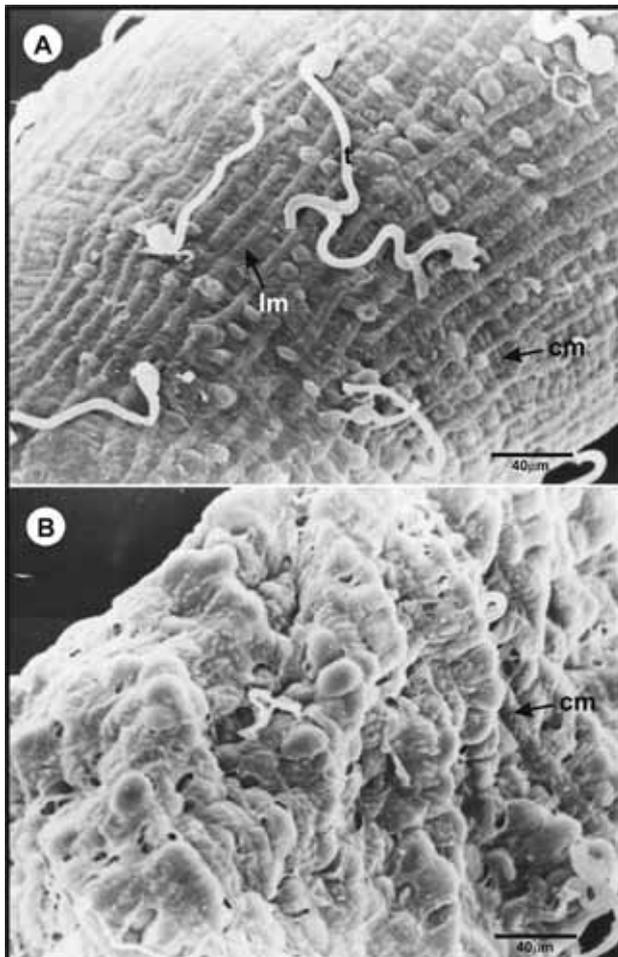


Figure 6. **A.** Detail of **Fig. 6B** showing the tracheal branch arriving between the muscle layers. **B.** Detail of the distal portion of the midgut showing its irregular appearance and the poorly defined circular muscle. **cm** - circular muscle layer, **lm** - longitudinal muscle layer, **t** - tracheal branch.

grooves of longitudinal fibers. A similar organization in which the outer surface consists of a layer of circular muscle has also been reported for the proventriculus of ants [9].

Caetano *et al.* [9] showed that longitudinal muscle fibers are located external to the layer of circular muscle in the midgut of ants, an arrangement similar to that seen in the two species studied here and in insects in general [10,22]. In *Solenopsis saevissima* (Formicidae, Hymenoptera), the midgut wall consists of three layers of muscle [2], whereas in *B. dorsalis* (Tephritidae, Diptera) there are only longitudinal fibers [16].

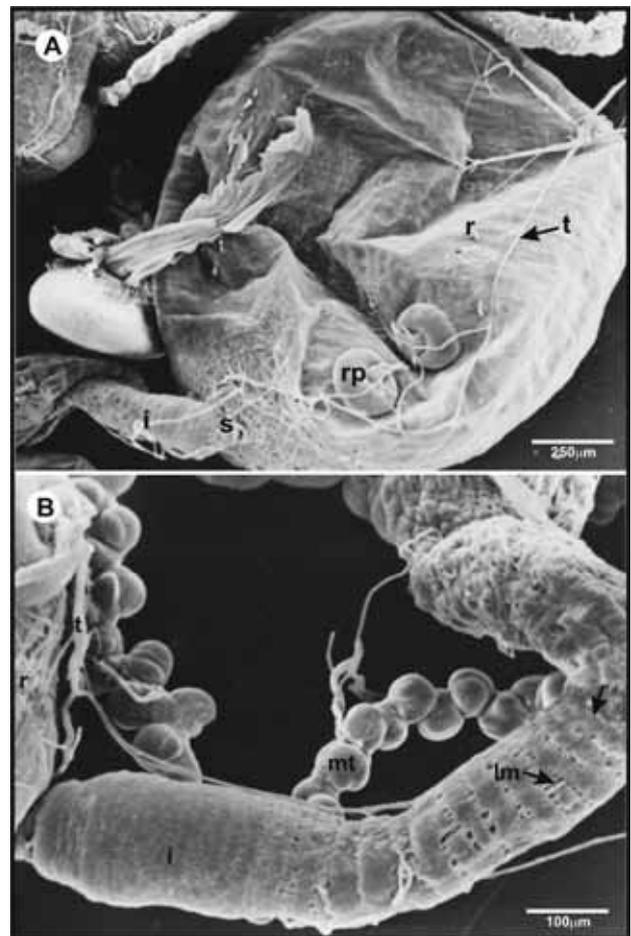


Figure 7. **A.** General view of the hindgut of a male *A. fraterculus* showing the ileum (**i**) and rectum (**r**). The anterior wall of the rectum has four rectal pads (**rp**) (only two are shown here) and a sphincter (**s**) is present where the ileum joins the rectum. Note the tracheal branch arriving at the rectal pads. **B.** General view of the ileum in a female *A. fraterculus*. Note the layer of circular muscle. The **arrow** indicates the pyloric region where the circular muscle of the ileum is more organized. **lm** - longitudinal muscle layer, **mt** - Malpighian tubules, **st** - posterior midgut, **t** - tracheal branch.

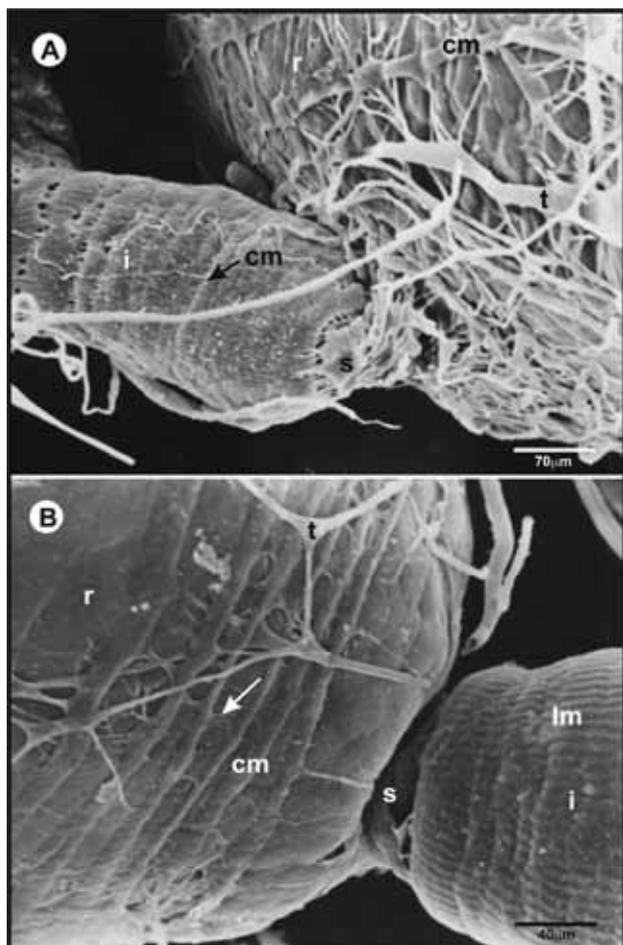


Figure 8. A. Detail of the sphincter at the ileal-rectal junction in a male *A. fraterculus*. Note the large number of tracheal branches (**t**) in this region and the strong circular muscle (**cm**) of the ileum and rectum. Detail of the same region in a female. Note the connective tissue fibers (**arrow**) that join the circular muscle fibers. **i** - ileum, **lm** - longitudinal muscle layer, **r** - rectum, **s** - sphincter, **t** - tracheal branch.

The ileum had a basic pattern that consisted of very well developed circular muscle and a sphincter at the beginning of the rectum; this organization was similar to that of most insects except for ants, in which the circular muscle is located between two layers of longitudinal fibers [7,8]. In some ants, particularly primitive species, the sphincter is located at the end of the ileum [8,9].

The rectum was much larger in *A. fraterculus* than in *C. capitata* but the circular muscle around this organ was less developed; similarly, the rectal pads in *A. fraterculus* were also 1.5 times larger than in *C. capitata*. These differences may be related to the size of the flies since adults of *C. capitata* are

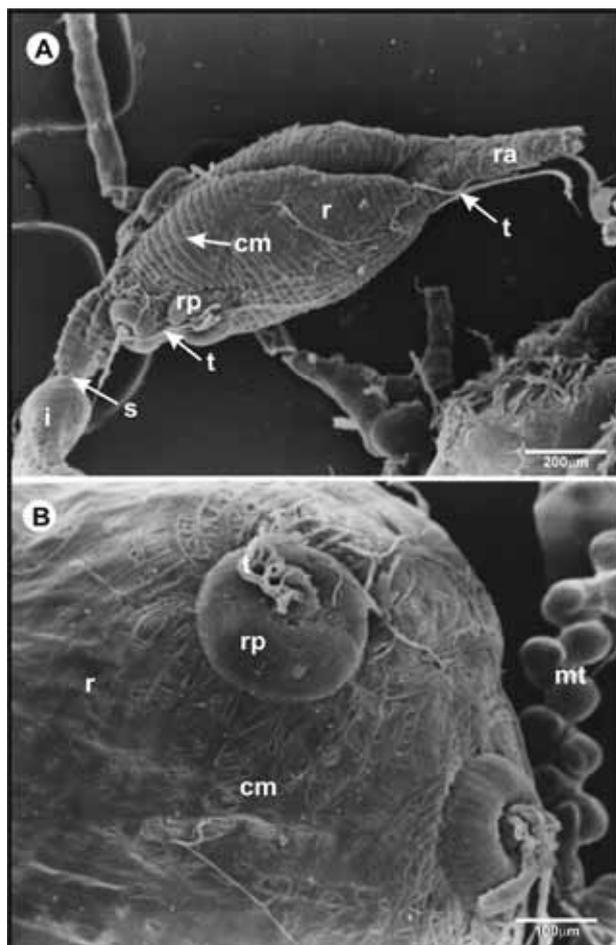


Figure 9. A. General view of the rectum in a female *C. capitata* showing the ileal-rectal sphincter, the strong circular muscle and the rectal pads (**rp**) in the anterior wall of the rectum. B. Detail of the same region of the rectum in *A. fraterculus* showing some slight depressions in the rectal pads. **cm** - circular muscle layer, **i** - ileum, **mt** - Malpighian tubules, **r** - rectum, **ra** - rectal-anal connection, **s** - sphincter, **t** - trachea.

smaller than those of *A. fraterculus*. The longitudinal striae of the rectal pads were more pronounced in *C. capitata* than in *A. fraterculus*. Variations in the size, shape and position of the rectal pads are common in ants, although the number of pads remains constant among various subfamilies [4,6,7,9]. However, overall, there is a trend towards a decrease in the size and number of rectal pads, from the non derivate to derivate subfamilies.

As in ants, the rectal pads of *A. fraterculus* and *C. capitata* had their own tracheal supply, although the number of tracheal branches was greater in these dipterans than in ants, in which only one tracheal branch reaches the rectal pads [9]. The rectum

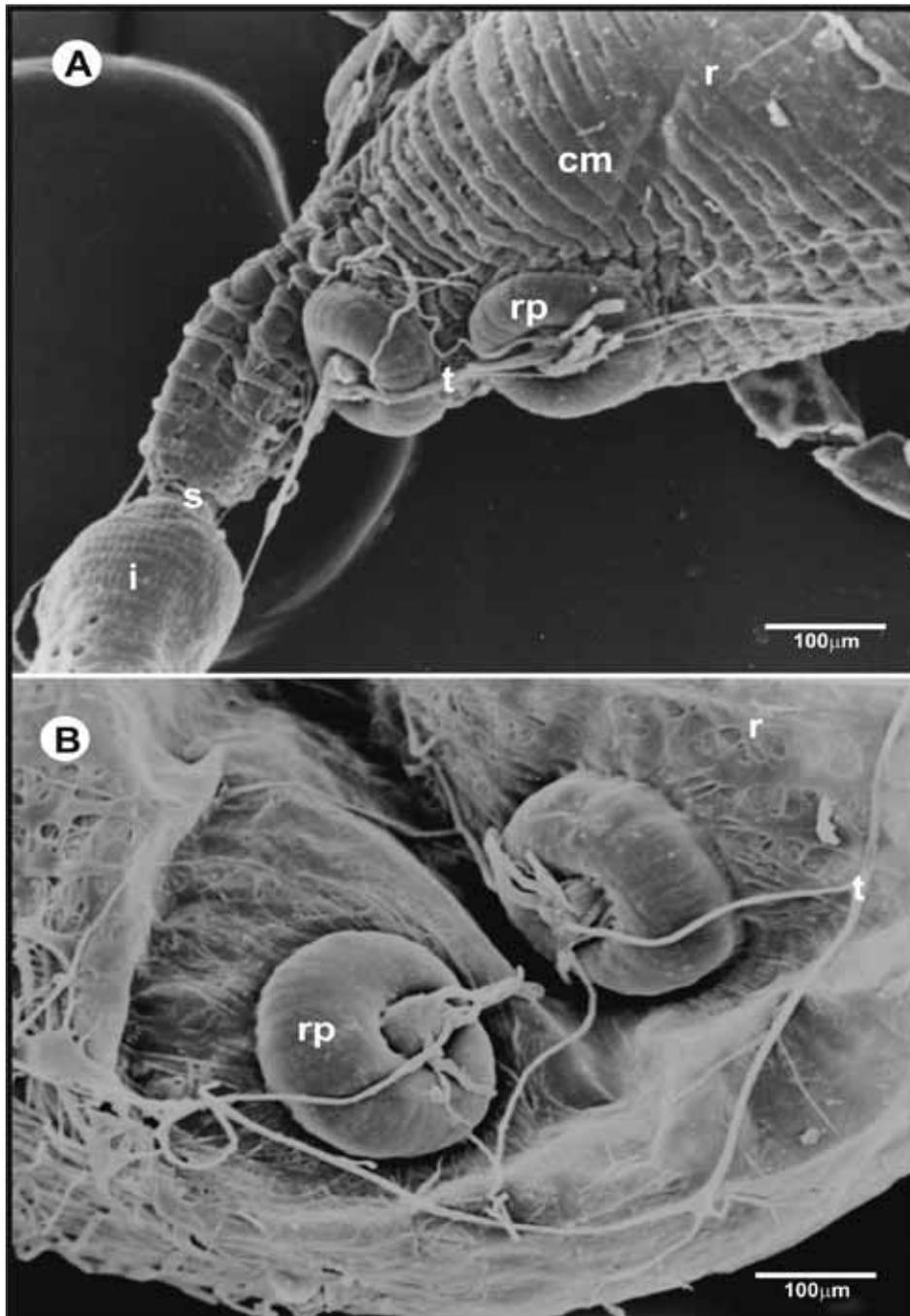


Figure 10. A and B. Comparison of the rectum in *A. fraterculus* and *C. capitata* showing the differences in the rectal muscle and size of the rectal pads. **cm** - circular muscle layer, **i** - ileum, **r** - rectum, **rp** - rectal pads, **s** - sphincter, **t** - trachea.

communicated with the external environment via the rectal-anal connection which, in ants, has a very well developed musculature and acts as a sphincter [9]. In the tephritids studied here, this region was divided into two parts and was connected to the cloaca.

The results of this study show that the anatomical organization of the digestive tract of tephritids is very

similar to that of other insects, although there are some important variations. We focused primarily on the external morphology of the digestive tract since the arrangement of the muscle layers may provide clues about the functions of this organ. Future studies should use histological and ultrastructural methods to investigate the internal organization of

the gut and its importance in sustaining symbiont bacteria involved in nitrogen fixation and as a probiotic diet [3,19], as well as its role in the release of male pheromones [18].

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