Postembryonic Development of Rectal Pads in Bees (Hymenoptera, Apidae)

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ABSTRACT

The morphology and development of the digestive tract of insects has been extensively studied, but little attention has been given to the development of the rectal pads. These organs are responsible for absorption of water and salts. In insects where they occur, there are usually six ovoid rectal pads located in the medial-anterior portion of the rectum. The rectal pad has three types of cells: principal, basal, and junctional. The arrangement of these three cell types delimits an intrapapillary lumen. The aim of the current study is to describe the development of the rectal pads during postembryonic development of *Melipona quadrifasciata anthidioides* and *Melipona scutellaris*. Specimens were analyzed at the following developmental stages: white-, pink-, brown-, and black-eyed pupae, and adult workers. The development of the rectal pad begins as a thickening of the epithelium in white-eyed pupae at 54 hr. At this stage, there is neither a basal cell layer nor intrapapillary lumen. The basal layers begin to form in the pink-eyed pupa and are completely formed at the end of the development of the brown-eyed pupa. In the brown-eyed pupal stage, the intrapapillary lumen is formed and the junctional cells are positioned and completely differentiated. Necrotic and apoptotic cell death were detected along with cell proliferation in the whole rectum during pupal development, suggesting that the development of the rectal pads involves cell proliferation, death, and differentiation. The rectal pads originate only from the ectoderm. Anat Rec, 292:1602–1611, 2009.

Key words: development; digestive tract; insects; stingless bees

The digestive tract of bees is divided into the foregut, midgut, and hindgut (Snodgrass, 1956; Serrão and Cruz-Landim, 1996, 2000; Serrão, 2001). The hindgut is a single layered epithelium of ectodermal origin comprised of different cell types covered by a cuticle (Cruz-Landim, 1994; Nation, 2002; Santos and Serrão, 2006).

The principal function of the hindgut is absorption of water and salt from the primary urine and the feces, thereby contributing to the osmotic control of the insect. Specialized structures for water and salt absorption can be found in the ileum of a few insect species, although,

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in most insects, specialized absorptive structures exist in the rectum, constituting the rectal glands (Wigglesworth, 1932; Cruz-Landim, 1994; Chapman, 1998). The principal site of water and electrolyte absorption is the rectum, which contains an epithelium with flattened cells, except in the area of the rectal pads, where the cells are cylindrical and have structural features that are associated with absorption, such as microvilli and basal plasma membrane infoldings associated with mitochondria (Chapman, 1998; Khodabandeh, 2006).

In bees, the rectal glands have two principal shapes: oval or elongated. Both can be either compact or hollow. The oval glands are termed rectal pads and they have a thickness similar to an epithelium. The elongated glands are conical infoldings of the epithelium into the lumen, and are called rectal papillae (Snodgrass, 1935; Ferreira and Cruz-Landim, 1969; Garayoa et al., 1999; Serrão et al., 2004).

In Hymenoptera, the rectal pads have three cell types: (1) the principal cells; (2) the tall and narrow junctional cells that sheath the principal cells and; (3) the basal cells that isolate the principal cells of the haemocoel (Noirot et al., 1979; Chapman, 1998). In honey bees (Apini) and stingless bees (Meliponini), the rectal pads have a layer of principal and two layers of basal cells (Snodgrass, 1956; Ferreira and Cruz-Landim, 1969).

Although the morphology and physiology of the rectum has been well studied (Berridge and Gupta, 1967; Ferreira and Cruz-Landim, 1969; Noirot and Noirot-Timothée, 1971; Nicolson and Louw, 1982; Bradley, 1985; Garayoa et al., 1999; Serrão et al., 2004), few works describe the development of the rectal pads during the postembryonic development.

The origin of the principal and basal cells in the rectal pads was investigated in pioneering studies with *Vespa vulgaris* (Evenius, 1933) and *Apis mellifera* (Lotmar, 1945; Dobrovsky, 1951); however, no consensus on the origin of these structures was developed. Evenius (1933) and Lotmar (1945) suggested that the two layers of basal cells would originate from free cells in the hemocoel. During postembryonic development, these cells would group under the principal cells to form the rectal pad, suggesting both a mesodermal and ectodermal origin. On the other hand, Dobrovsky (1951) suggested an ectodermal origin for the rectal pads, because the basal cells arise from the rectal epithelium, along with the principal cells, during the pupal stage. In an extensive study on metamorphosis in the digestive tract of the stingless bee *Melipona quadrifasciata anthidioides*, Cruz-Landim and Mello (1970) were unable to conclude whether rectal pads had an ectodermal or mesodermal origin.

The objective of the current is to describe the ontogeny of the rectal pads during postembryonic development in the stingless bees *Melipona scutellaris* and *Melipona quadrifasciata anthidioides*, to gain new insight on the origin of rectal pads in insects.

MATERIAL AND METHODS

Animals

Different developmental stages (larva, pupa and adult) of *Melipona scutellaris* and *Melipona quadrifasciata anthidioides* workers were obtained from colonies housed in the Central Apiary of the Federal University of Viçosa, Minas Gerais, Brazil, from August 2007 to April 2008. Pupae were divided into four developmental stages according eye pigmentation: white-, pink-, brown-, or black-eyed pupae.

On the basis of the preliminary results, brood combs containing fourth instar larvae were collected directly from the colonies, maintained in petri dishes at 28°C, and observed daily for determination of the start of pupal stages as characterized by larval moult. After ecdysis, white-eyed pupae were dissected at 6-hr intervals for a total period of 96 hr of development. Pink-eyed pupae were also maintained in petri dishes at 28°C and dissected at 24-hr intervals for a total of 72 hr of development. Larvae, brown- and black-eyed pupae were obtained directly from the brood combs and adults from the nest.

Histology

For histological analyses, the bees were cryo-anesthetized and dissected in a 125 mM NaCl solution. The rectums were transferred to Zamboni's solution (Stefanini et al., 1967) for 24 hr, dehydrated in a graded ethanol series, embedded in resin (Historesin, Leica Microsystems, Nussloch, Germany), and cut in 4-μm thin slices. The samples were stained with hematoxylin and eosin.

Histochemistry

Rectums from white-, pink-, brown-, and black-eyed pupae were submitted to the Feulgen reaction to identify DNA and processed for histological analysis. The 5-μm thin sections were counter-stained with 10% light green (Pearse, 1953).

Detection of Cell Proliferation

Cell proliferation was investigated using an anti-bromodeoxyuridine (BrdU) kit (Boehringer-Mannheim, Mannheim, Germany). Rectums of white-eyed pupae were dissected in 0.1 M sodium phosphate buffer (PBS) and incubated with 0.2 mg/mL BrdU in PBS plus 1% Tween-20 (PBST) for 50 min (Baker and Yu, 2001). The samples were then transferred to Zamboni's fixative solution for 15 min, washed in PBS for 1 hr, followed by incubation in anti-BrdU (1:100) in buffer for 24 hr at 4°C (Boehringer-Mannheim). The samples were washed three times in PBS, and incubated with a FITC-conjugated anti-mouse IgG secondary antibody for 24 hr at 4°C. The samples were washed in PBS, dehydrated in a graded ethanol series, embedded in resin and the 10-μm thin slices were mounted on slides with 50% sucrose, and analyzed by an Olympus BX60 fluorescence microscope with a WB filter. The negative control was obtained by the omission of the anti-BrdU antibody.

Detection of Programmed Cell Death

Cell death was detected using an *in situ* cell death detection kit (TUNEL™, Roche Diagnostics Corp., Indianapolis, IN). Rectums of white- and pink-eyed pupae were dissected in 125 mM NaCl, transferred to 4% paraformaldehyde, and sectioned on a cryostate (LEICA CM1850). The 10-μm thin sections were submitted to the TUNEL protocol according to manufacturer's instructions using a peroxidase-conjugated antibody. Negative controls were obtained by incubation in solution without

the terminal transferase enzyme. The slices were counter-stained with 10% light green and analyzed by light microscopy.

Scanning Electron Microscope

The rectums from white-, pink-, brown-, and black-eyed pupae and from adult workers were dissected in 125 mM NaCl, fragmented to expose the luminal face and transferred into 2.5% glutaraldehyde in sodium cacodylate buffer. The samples were postfixed in 1% osmium tetroxide in the same buffer. The tissue fragments were transferred to ethanol and submitted to CO_2 critical point drying (CPD Baltec 30, Leica Microsystems GmbH, Wetzlar, Germany), coated with gold (20 nm), and analyzed in a scanning electronic microscope LEO VP 1430 (Carl Zeiss, Jena, Germany).

RESULTS

Similar results for rectal pad development were observed in both *M. quadrifasciata anthidioides* and *M. scutellaris*. The rectum of adult workers has a single layered epithelium, covered internally by a thick cuticle and, externally, involving a layer of inner circular and outer longitudinal muscles. The epithelial cells of the rectal wall are flattened with circular nuclei, which present clusters of condensed chromatin in *M. scutellaris*. The six rectal pads are located in the medial-anterior portion of the rectum. Each rectal pad is composed of three cell types: principal, basal (or secondary), and junctional cells (Fig. 1A–C). The layer of principal cells delimits the rectal lumen, which is covered by a thinner cuticle than that covering the rectal epithelium. These cells are cylindrical with a medial-basal nucleus. The basal cells are cubical with round nuclei, constituting two cell layers that are isolated from the principal cells with an intrapapillary lumen and a basal portion that is closely associated with the muscle layer that recovers the whole rectum (Fig. 1B,C). The junctional cells are narrow with elongated nuclei and they mark the transition between the rectal epithelium and the principal cells. Although the basal layers are not in direct contact with the rectal epithelium, they are continuous with the junctional cells (Fig. 1C).

The larval rectum is a mostly undifferentiated organ that is similar to an elongated tube with a thick, stratified epithelium. The cuticle is thin, poorly developed and difficult to visualize. Small chromatic bodies, which are stained strongly by hematoxylin, are found scattered throughout the entire epithelium (Fig. 1D).

In the early white-eyed pupae, the rectal epithelium is thick, but without the stratified aspect of the larvae, with a thin cuticle that does not cover the entire epithelium (Fig. 1E). The chromatic bodies are rare at this stage.

In 30-hr-old white-eyed pupae, the rectum shows enlarged size and volume and it is easily distinguished from the ileum (Fig. 1F). The chromatic bodies reappear along the epithelium, having been released from the nuclei of epithelial cells (Fig. 2A). These structures are Feulgen-positive, providing evidence for the presence of DNA (Fig. 2B). The epithelial cells are cubical and frequently observed within mitosis. The occurrence of cell proliferation was also shown by presence of BrdU-positive cells (Fig. 2C). The cuticle is more evident than in the early white-eyed pupae; however, it is weakly associated with the cell apex and is sometimes removed during dissection.

In the white-eyed pupae at 54 hr, the rectal pads are observed as a thickening of the rectal epithelium, with a layer of principal cells constituting a stratified epithelium. The basal cells are located under the principal cells; they are small and not organized into different layers (Fig. 2D). Chromatic bodies are present in the rectal epithelium and in the developing rectal pads. The junctional cells are absent, and there is no evident separation between the pads and the rectal epithelium. The cuticle does not cover all of the epithelia cells (Fig. 2F). Between 54 and 96 hr of white-eyed pupal development, the shape of the rectal pads changes from elongated to disc-shaped, as seen in pink-eyed pupae (Fig. 2E).

In the pink-eyed pupae, the six rectal pads can be differentiated from the rectal epithelium, although they are still undergoing differentiation (Fig. 3A,B). In 24-hr-old pink-eyed pupae, chromatic bodies are found in the principal cells, in the area that will arise in the intrapapillary lumen (Fig. 3B), as well as in the rectal epithelium. The junctional cells begin the differentiation process and are located in the transition area between the rectal pads and the rectal epithelium after 48 hr of development. Differentiation of these cells is characterized by the presence of elongated and narrowed nuclei. The rectal epithelium is evenly populated by cubical cells, while the principal cells are columnar (Fig. 3C). The basal cells are not yet organized into two layers. The number of chromatic bodies is reduced in the later developmental stages of the pink eyed-pupae, along with an observed increase in the number of vacuoles inside the principal cells (Fig. 3C,D,F) and the occurrence of TUNEL-positive cells (Fig. 3E). The thickness of the rectal epithelium begins to decrease, and many vacuoles are observed in the apical area of these cells (Fig. 3C,F). Chromatic bodies and mitosis are observed in the cells of the rectal epithelium as well as in the rectal pads (Fig. 3D).

In brown-eyed pupae, the rectal pads have a disc-like shape and the basal cells are organized in two layers (Figs. 3F, 4A). The principal cells form a single layer. The central portion of the rectal pads has vacuoles and the intrapapillary lumen begin to develop. Small cells with vacuolated cytoplasm and pycnotic nucleus (Fig. 4C) are observed in regions where the intrapapillary lumen is already formed. These cells are in contact with the basal cells. The junctional cells are well developed (Fig. 4B) and they isolate the principal cells from the rectal epithelium.

Black-eyed pupae have rectal pads with an aspect similar to those found in adult workers, with columnar principal cells, two layers of basal cells, and junctional cells that form a collar around the principal cells. The principal cells possess elongated nuclei and vacuoles in the basal portion. The intrapapillary lumen is completely formed, but with some small cells similar to those found in the brown-eyed pupae (Figs. 4D,E). The rectal epithelium shows flattened cells and folds like found in adult bees (Fig. 4F).

Synthesis of the cuticle can be observed during rectal pad differentiation. Synthesis began in the white-eyed pupae and in the pink-eyed pupae it appears as a

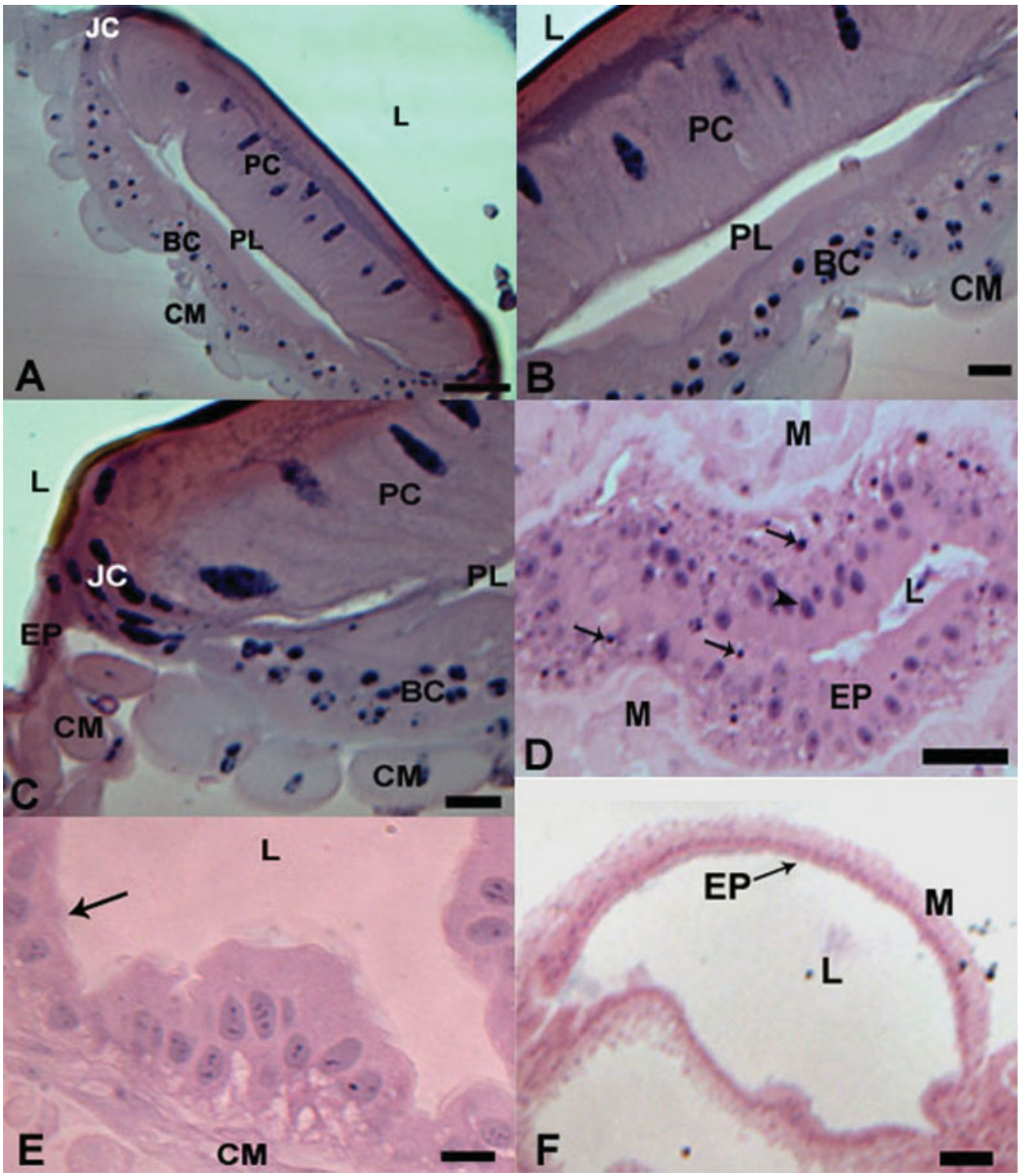

Fig. 1. Longitudinal section of the rectum of *Melipona scutellaris* (**A**–**C**) and *Melipona quadrifasciata anthidioides* (**D**–**F**). A: Rectal pad of adult worker.PC, principal cells; BC, basal cells; JC, junctional cells; PL, intrapapillary lumen; L, lumen; CM, circular muscle. Bar: 20 μm. B: Rectal pad of adult worker with principal cells (PC) and two layers of basal cells (BC). PL, intrapapillary lumen; CM, circular muscle; L, lumen. Bar: 10 μm. C: Junctional cells (JC) of adult workers between principal cells of the rectal pad (PC) and the rectal epithelium (EP). BC, basal cells; L, lumen; PL, intrapapillary lumen; CM, circular muscle. Bar: 10 μm. D: Rectal epithelium (EP) of larva showing a stratified aspect with some chromatic bodies (arrows). L, lumen; M, muscle; arrowheads, nucleus. Bar: 10 μm. E: Rectum of early white-eyed pupae showing the epithelium with tall cells covered by a thin cuticle (arrow). L, lumen; CM, circular muscle. Bar: 10 μm. F: Rectum of white-eyed pupae 36-hr-old showing a sac-like aspect. EP, epithelium; L, lumen; M, muscle. Bar: 100 μm. Light microscope.

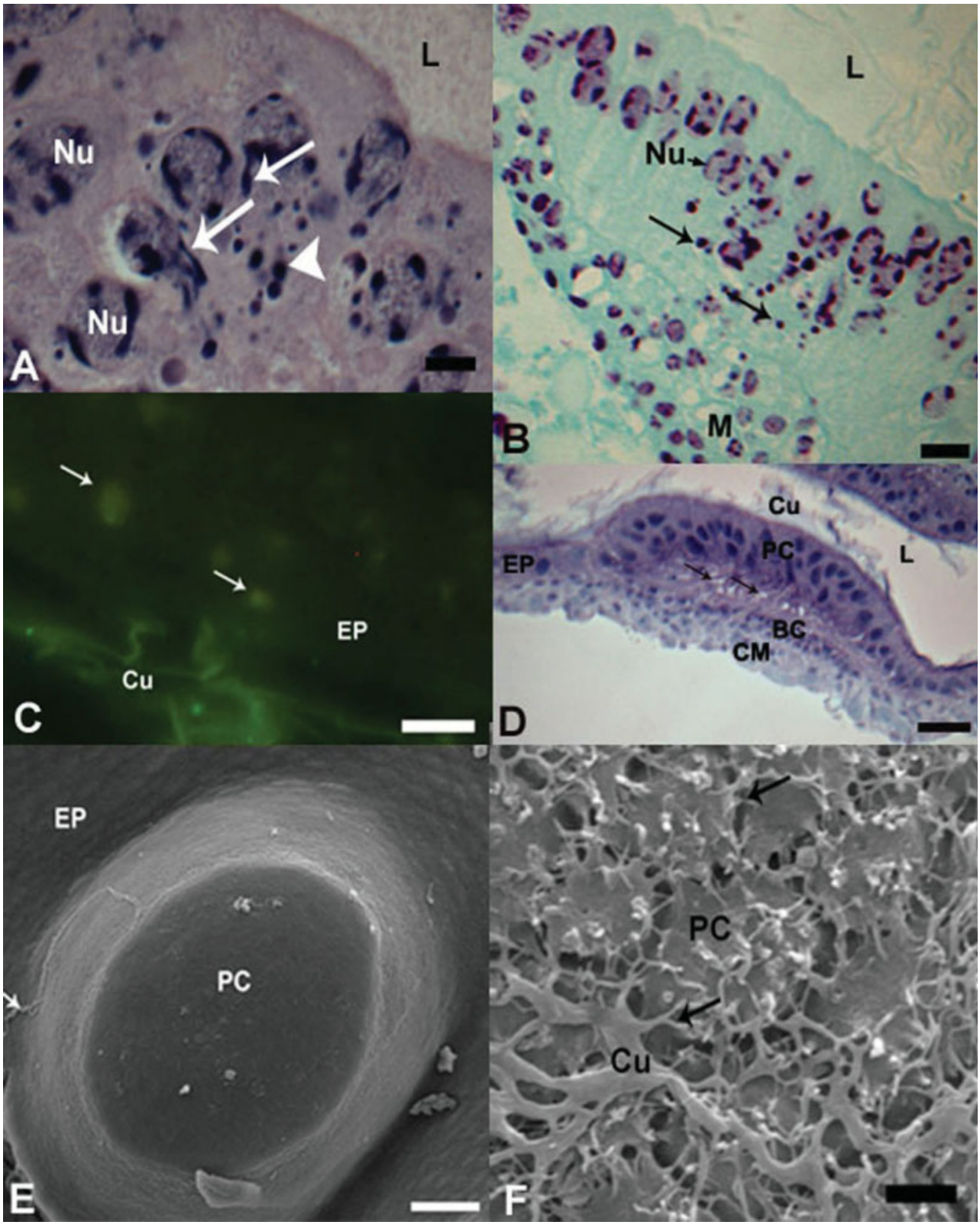

Fig. 2. White-eyed pupae rectum. **A:** *Melipona scutellaris* white-eyed pupae showing nucleus (Nu) of epithelial cells fragmented (arrows) releasing the chromatic bodies (arrowheads). L, lumen. Bar: 10 μm. **B:** *Melipona scutellaris* white-eyed pupae showing nucleus (Nu) of epithelial cells and chromatic bodies (arrows) Feulgen-positives. L, lumen; M, muscle. Bar: 10 μm. **C**: *Melipona scutellaris* white-eyed pupae showing cell BrdU positives (arrows) in the epithelium (EP) and the autofluorescence of the cuticle (Cu). Bar: 10 μm. **D:** *Melipona quadrifasciata anthidioides* white-eyed pupae 54–hr-old, showing the principal cells (PC) of the rectal pad with stratified aspect and the basal cells (BC) widespread along the rectal pad. Arrows, vacuoles; CM, circular muscle; Cu, cuticle; L, lumen; EP, epithelium. Bar: 20 μm. **E**: Scanning electron micrograph of the rectum of *Melipona quadrifasciata anthidioides* white-eyed pupae showing disc-shaped rectal pad. EP, epithelium; PC: principal cells. Bar: 20 μm. **F:** Scanning electron micrograph of the rectum of *Melipona quadrifasciata anthidioides* white-eyed pupae 66-hr-old showing the cuticle (Cu) with a network aspect (arrow). PC, principal cells. Bar: 2 μm. Light microscope (A,B,D), Fluorescence microscope (C).

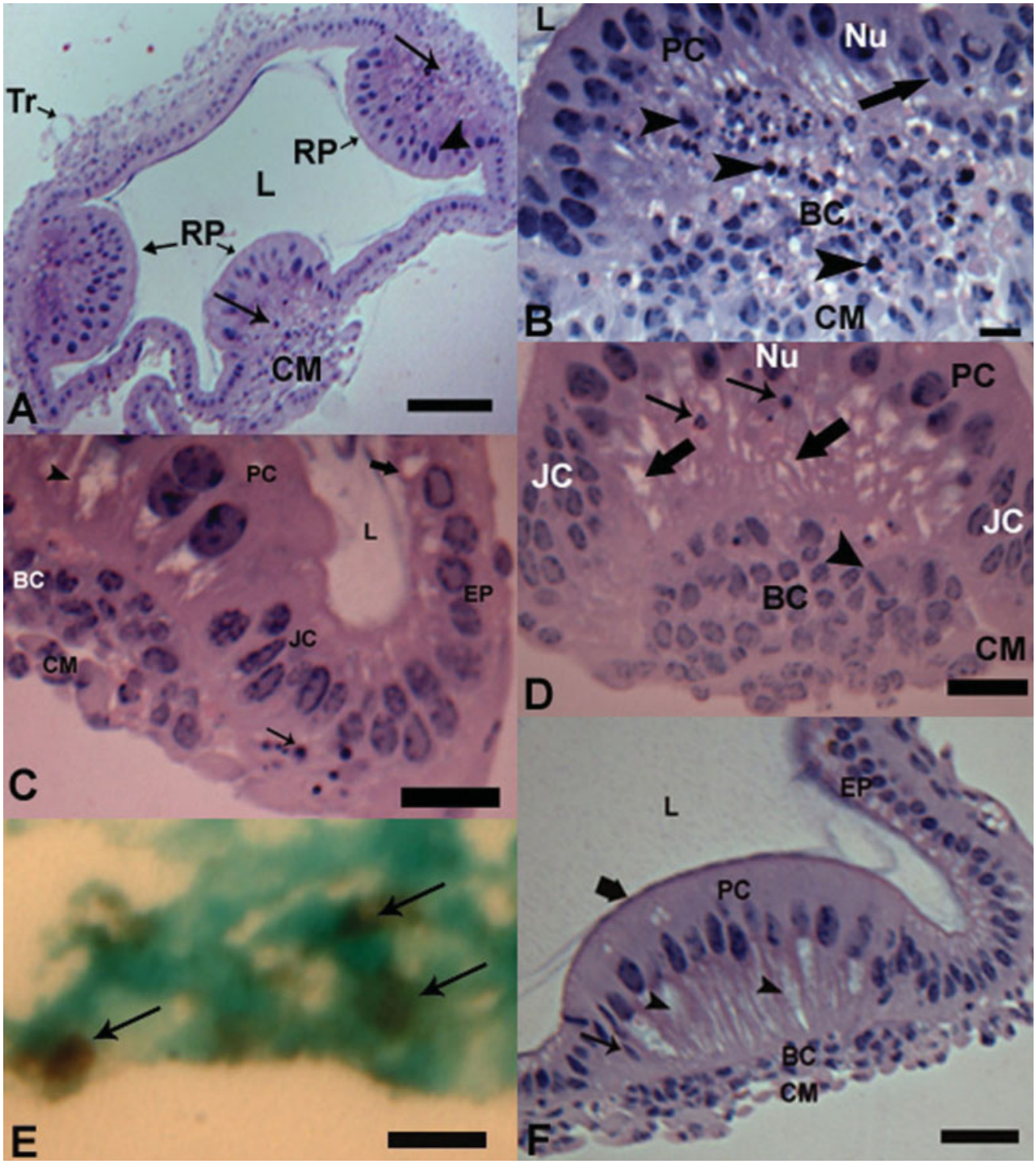

Fig. 3. Pink and brown-eyed pupae rectum. **A:** *Melipona quadrifasciata anthidioides* pink-eyed pupae showing three rectal pads (RP). L, lumen; CM, circular muscle; arrow, chromatic bodies; arrowhead, nucleus principal cells; Tr, trachea. Bar: 50 μm. **B:** *Melipona scutellaris* pink-eyed pupae 24-hr-old showing differentiation of the junctional cells (arrow) and basal cells (BC) of the rectal pad. Note chromatic bodies (arrowheads) closely to principal cells (PC). L, lumen; CM, circular muscle. Bar: 10 μm. **C:** *Melipona scutellaris* pink-eyed pupae 72-hr-old showing epithelial cells (EP) with vacuoles (large arrow), which are larger (arrowheads) in the central region of the rectal pad. BC, basal cells; PC, principal cells; JC, junctional cells; L, lumen; CM, circular muscle; arrow, chromatic body. Bar: 20 μm. **D:** *Melipona scutellaris* pink-eyed pupae 72-hr-old showing mitosis (arrowhead) in the basal cells (BC). The chromatic bodies (arrow) are rare, whereas vacuoles are large (large arrow). PC, principal cells; JC, junctional cells; CM, circular muscle; Nu, nucleus. Bar: 20 μm. **E:** *Melipona scutellaris* pink-eyed pupae showing Tunnel positive nuclei (arrows). Bar: 10 μm. **F:** *Melipona scutellaris* brown-eyed pupae showing principal cells (PC) arrayed in a single layer. Note the presence of large vacuoles (arrowheads) and basal cells (BC) arranging in layers. Arrow, junctional cell; CM, circular muscle; EP, epithelium; Large arrow, cuticle; L, lumen. Bar: 10 μm. Light microscope (A–F).

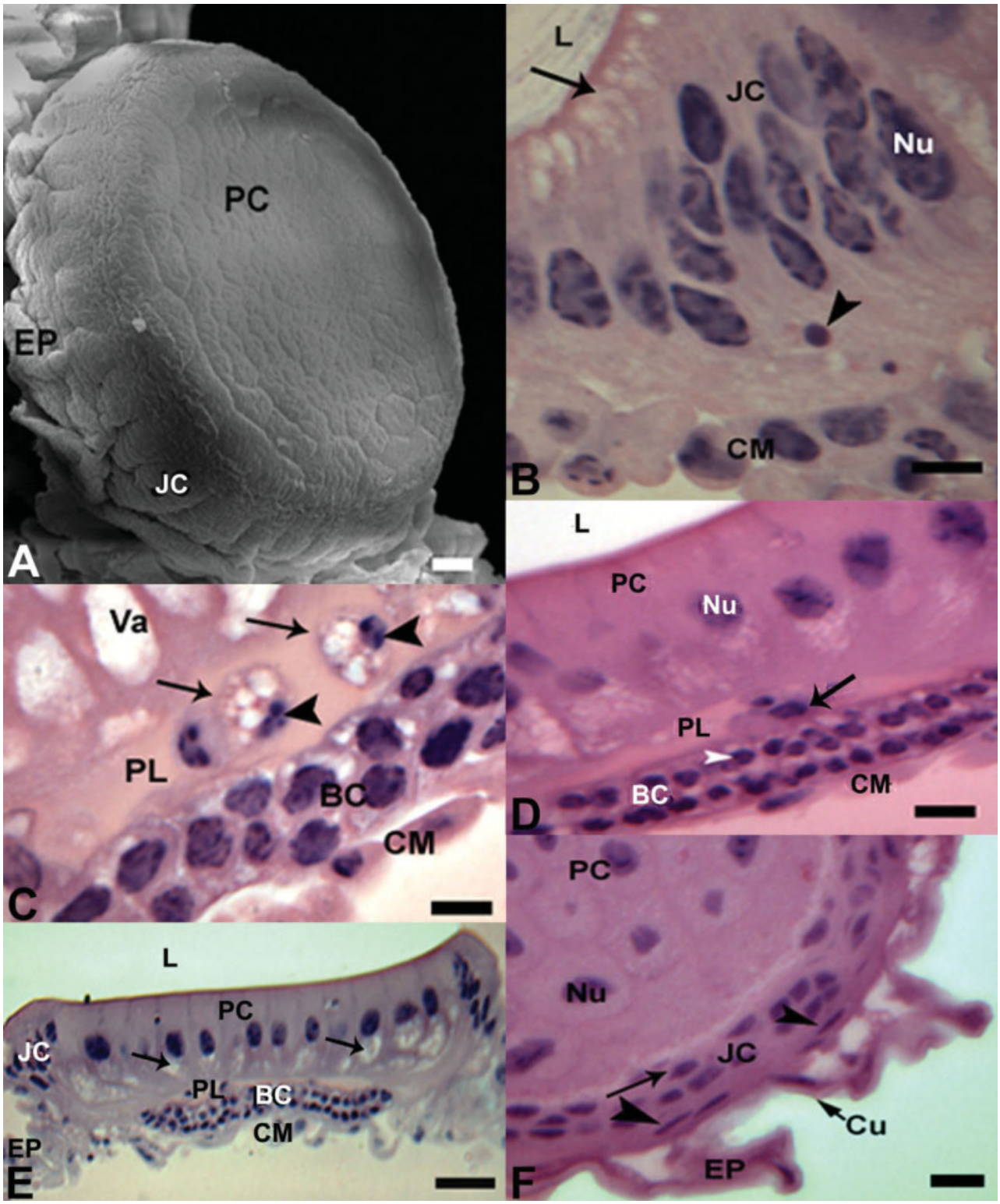

Fig. 4. Brown and black-eyed pupae rectum. **A:** Scanning electron micrograph of the rectum of *Melipona quadrifasciata anthidioides* brown-eyed pupae showing a disc-like rectal pad with impressions of the principal cells (PC) in the cuticle and the folded rectal epithelium (EP). JC, junctional cells. Bar: 10 μm. **B:** Section of the *Melipona scutellaris* brown-eyed pupae rectum showing junctional cells (JC) with narrowed cytoplasm, nucleus (Nu), and apical vacuoles (arrow). L, lumen; CM, circular muscle; arrowhead, chromatic body. Bar: 10 μm. **C:** Section of the *Melipona scutellaris* brown-eyed pupae rectum showing the intrapapillary lumen (PL) containing cells (arrow) with vacuolated cytoplasm and pycnotic nucleus (arrowhead). BC, basal cells; CM, circular muscle; Va, vacuoles. Bar: 10 μm. **D:** Section of the rectum of *Melipona scutellaris* black-eyed pupae showing cells (arrow) into the intrapapillary lumen (PL) and cubic basal cells (BC) with rounded nucleus (arrowhead). PC, principal cells, CM; circular muscle; L, lumen; Nu, nucleus. Bar: 10 μm. **E:** *Melipona scutellaris* black-eyed pupae rectal pad with the same features present in the adult one. Arrows, vacuoles; BC, basal cells; CM, circular muscle layer; EP, epithelium; JC, junctional cells; L, lumen; PC, principal cells; PL, intrapapilary lumen. Bar: 20 μm. **F:** Transversal section of the rectum of *Melipona scutellaris* black-eyed pupae showing junctional cells (JC) isolating the principal cells (PC) from the rectal epithelium (EP). Cu, cuticle; Nu, nucleus of principal cells; arrow, nucleus of junctional cells; arrowheads, nucleus of epithelial cells. Bar: 10 μm. SEM (A), Light microscope (B-F).

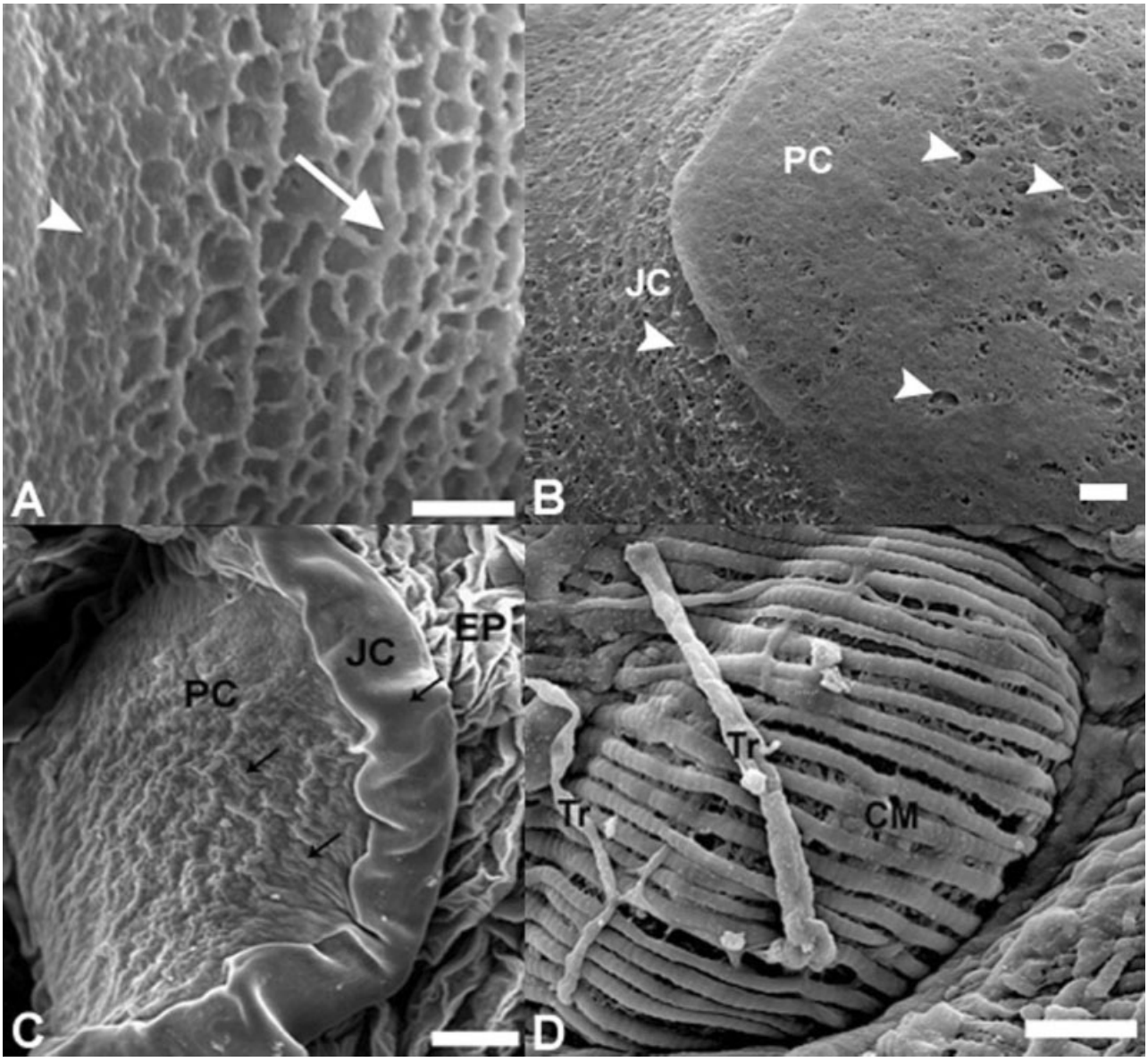

Fig. 5. Scanning electron micrographs of the rectum of *Melipona quadrifasciata anthidioides*. **A:** Pink-eyed pupae with cuticle covering the junctional (arrow) and epithelial cells (arrowhead). Bar: 2 μm. **B:** Brown-eyed pupae showing the cuticle that covers the principal (PC) and junctional cells (JC) with hollows (arrowheads). Bar: 3 μm. **C:** Black-eyed pupae showing disc-like rectal pad. Note the cuticle aspects (arrows) present onto the principal (PC) and junctional cells (JC). EP, folded rectal epithelium. Bar: 20 μm. **D:** Black-eyed pupae showing the basal region of the rectal pad covered only by ciruclar muscles (CM) with associated trachea (Tr). Bar: 20 μm.

network aspect in the lateral area of the rectal pad (Fig. 5A). In a posterior region, the cuticle forms a uniform layer covering the entire rectal epithelium (Fig. 5B,C). At that time the pads are similar to those found in adult bees (Fig. 5D).

DISCUSSION

During the larval development of *Melipona quadrifasciata anthidioides* and *Melipona scutellaris*, the epithelium of the hindgut (ileum and rectum) is composed of tall cells that are arrayed in several layers in the rectum, similar to what was observed by Cruz-Landim (2004). However, Cruz-Landim and Mello (1970) and Cruz-Landim (2004) state that rectal pad differentiation occurs in pink-eyed pupae, whereas we find that differentiation of the rectal pads begins in white-eyed pupae at 54 hr.

The chromatic bodies, found in the rectal epithelium and rectal pads in all pupal periods analyzed, contain some DNA and cytoplasmic remnants. In *A. mellifera*, Dobrovsky (1951) found chromatic bodies distributed in six regions of the anterior portion of the rectum; these were the areas where the rectal pads would later develop. However, the distribution and occurrence of the chromatic bodies in the species analyzed in this study

differs from that described by Dobrovsky (1951). We suggest that the role of the chromatic bodies is not directly related to the formation of the rectal pads. Supporting this idea is the fact that chromatic bodies are present in pupae that already have differentiated rectal pads, and they also occur in the rectal epithelium.

The discovery of cell proliferation and cell death during differentiation of the rectum suggests that the morphology of this organ in adult bees results from epithelial remodeling during the pupal stage. The presence of TUNEL-positive cells, and cells with vacuolated cytoplasm and pycnotic nuclei within the intrapapillary lumen may also be indicative of the occurrence of cell death (Kerr et al., 1972; Souza et al., 2007). In the insect gut, both apoptotic and necrotic cell death have been observed (Rost, 2006; Tettamanti et al., 2007; Rost-Roszkowska, 2008a, 2008b; Fialho et al., 2009; Park et al., 2009), while in some species necrosis accompanies apoptosis (Rost-Roszkowska, 2008b). Cytoplasmic vacuolization is strong evidence of cell necrosis, which is followed by cell rupture and discharge of the contents into the gut lumen (Rost-Roskowska, 2008b). On the other hand, apoptosis is characterized by cell shrinkage, chromatin condensation and nuclear fragmentation, followed by the discharge of apoptotic bodies into the gut lumen (Rost, 2006; Tettamanti et al., 2007; Rost-Roskowska, 2008b). Thus, chromatic bodies would not be rectal pads precursors, but fragments of dead, likely apoptotic, cells. This is supported by the presence of TUNEL-positive cells in the rectal pads of *Melipona quadrifasciata anthidioides* pupae.

In addition to cell proliferation and cell death during differentiation of the rectal pads there is also cellular differentiation, especially of basal and junctional cells. The rectal pads are constantly remodeled from a solid, elongated structure in white-eyed pupae to a disc-like structure with a lumen in black-eyed pupae as a result of the interaction between those three events—cell proliferation, death, and differentiation.

Evenius (1933) suggested a mesodermal origin for basal cells of the rectal pads, which would migrate from the hemocoel and cross the muscle layers during the differentiation process. Cruz-Landim and Mello (1970) suggested that Evenius' hypothesis might be correct because, in some adult bees lacking rectal pads (Ferreira and Cruz-Landim, 1969), mesodermal cells accumulate near the rectum. On the other hand, Dobrovsky (1951) suggested an ectodermal origin for rectal pads. Our findings suggest that Dobrovsky's theory is correct, because both the principal and basal cells arise from cells already present in the rectal epithelium. In all histological sections analyzed, mesodermal cells were never seen to accumulate near the rectal wall, nor was there evidence of cell migration from the hemocoel to the rectum. In young pupae (54-hr-old white-eyed pupae), there is a cluster of small cells at the basal region of the rectal wall with an intact muscle layer below. In the brown-eyed pupae, cells that are initially grouped undergo proliferation and migration to give rise to the two basal layers of the rectal pads.

Differentiation of the rectal pads occurs during the entire pupal stage of the bee. Although rectal pads with the same aspect as those in adult bees can be found in black-eyed pupae, the presence of cell fragments in the intrapapillary lumen of the rectal pads indicates the final stages of the differentiation process for this structure.

The rectal pads, along with the entire rectal epithelium, are covered by a cuticle synthesized by epithelial cells, with the fibrils being released in clusters. There are morphological differences between the cuticles that cover the principal and junctional cells of the rectal pads and the rectal epithelium. The principal cells are the main site of water and ion absorption. They contain ultrastructural characteristics that are associated with that function, such as a complex system of intercellular spaces and basal plasma membrane infoldings associated with the mitochondria (Berrige and Gupta, 1967; Jarial, 1992; Garayoa et al., 1999; Serrão et al., 2004). The cuticle that covers the principal cells has a less homogeneous appearance than that found in the ring of junctional cells and in the rectal epithelium. During differentiation of the rectal pads, there is a thickness to the cuticle that covers the junctional cells, which is released like a net that later will assume a homogeneous aspect. There is continuity among the different cuticle types, such as is found in ants, even though they have different characteristics (Garayoa et al., 1999).

During postembryonic development of bees, the rectum changes from a tube-like structure in the larva to an enlarged sac in the adult. The increase in rectal volume begins during the white-eyed pupal stage due to the cell proliferation and changes in the morphology of the epithelial cells of the rectum wall. Simultaneously with the development of the rectal pads, the rectal epithelium loses its stratified aspect from cylindrical cells with a decrease in cell size to generate the flattened cells found in adult bees (Cruz-Landim, 2004).

The rectal pads begin to differentiate in the white-eyed pupa 54 hr after the beginning of the pupal stage and this involves the processes of cell proliferation, death, and differentiation. The rectal pads arise from the rectal epithelium with an ectodermal origin and their differentiation is continuous during the entire development of the pupa.

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