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Post-embryonic Development of Intramandibular Glands in *Pachycondyla verenae* (Forel) (Hymenoptera: Formicidae) workers

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Abstract

The current knowledge of intramandibular glands in Hymenoptera is focused on occurrence and morphology in adult insects. This is the first report regarding the post-embryonic development of intramandibular glands in a "primitive" ant, *Pachycondyla verenae*. In this study, we analyzed mandibles of prepupae, white-eyed, pink-eyed and black-eyed pupae, pupa of pigmented body pupae, and adults. Adult workers of *P. verenae* have intramandibular glands with epidermal secretory cells of class I and isolated glands of class III, and both glands have onset differentiation in pink-eyed pupae. Some histological sections were submitted to histochemical test for total proteins and neutral polysaccharides. Histochemical tests showed occurrence of polysaccharides and proteins in epidermal secretory cells of class I from the white-eyed pupae, polysaccharides and proteins in pink-eyed pupae to black-eyed pupae in both glands classes I and III and presence of polysaccharides in adult ants also in both gland classes I and III. Intramandibular glands of classes I and III in *P. verenae* workers differentiate during pupation, with onset occurring in pink-eyed pupae, and completion occurring in black-eyed pupae.

Introduction

Hymenoptera have two mandibular gland types, the ectomandibular or mandibular glands, and the mesomandibular or intramandibular glands (Cruz-Landim & Abdalla, 2002). Unlike the mandibular glands that are the most studied, the current knowledge regarding intramandibular glands is restricted to occurrence and descriptive morphology in adult bees (Nedel, 1960; Toledo 1967; Costa-Leonardo, 1978; Cruz-Landim & Abdalla, 2002; Santos et al. 2009a, Cruz-Landim et al., 2011) and ants (Schoeters & Billen, 1994, Billen & Espadaler, 2002, Grasso et al., 2004, Roux et al., 2010, Martins & Serrão, 2011).

The intramandibular glands in ants can be divided in three classes (Martins & Serrão, 2011). First, gland characterized by cubical or columnar epidermal cells, which are described as class I gland cells by Noitot & Quennedey (1974). Second, isolated glands with spherical cells in the inner cavity of the mandible characterized by the presence of canaliculi that open in pores on the mandible surface, this gland class has a secretory and a conducting cell (Billen 2009) and is classified as class III gland cells by Noitot & Quennedey (1974). The third type occurs in Attini, with hypertrophy of epidermal cells containing a reservoir (Amaral & Caetano, 2006; Billen, 2009; Martins & Serrão, 2011).

In ants, studies of salivary system are restricted to morphology of post-pharyngeal and hypopharyngeal (Gama, 1978; Amaral & Caetano, 2005), mandibular (Gama, 1978; Pavon & Camargo-Mathias, 2005;), labial or salivary glands (Gama, 1978; Meyer et al., 1993; Lommelen, et al., 2002; 2003; Amaral & Machado-Santelli, 2008) and intramandibular glands (Martins & Serrão, 2011) but the intramandibular glands development remains unclear.

Ponerini ants are an interesting model to study gland development since their representatives have features similar to ants ancestor (Kusnezov, 1955; Peeters & Crewe, 1984, Hölldobler & Wilson, 1990).

In this work we describe the histology and histochemistry of intramandibular glands from prepupae to adult workers of the Neotropical ant *Pachycondyla verenae*.

Material and Methods

Five colonies of *P. verenae* were collected in the experimental field of the Department of Fruit Science, Federal University of Viçosa, Viçosa, state of Minas Gerais, Brazil. Worker ants at the following developmental stages were evaluated: prepupae, white-eyed, pink-eyed and black-eyed pupae, body pigmented pupae, and adults (Fig. 1A, 1B). Features that allowed for the identification of the successive post-embryonic developmental stages were the focus of these evaluations (Soares et al., 2004).

Five individuals in each developmental stage were -anesthetized by thermal shock at 0 ^{o}C for 3 min and the mandibles were removed and transferred to Zamboni fixative solution (Stefanini et al., 1967). Mandibles were dehydrated in a graded ethanol series, embedded in JB-4 historesin, and 3 µm slices were stained with hematoxylin and eosin.

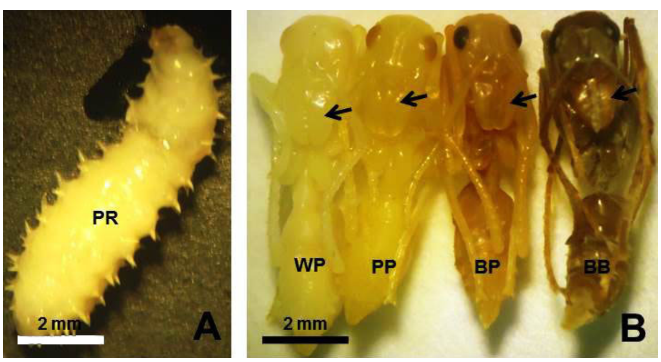

Fig. 1: Different post-embryonic developmental stages in *Pachycondyla verenae* workers. A - prepupae (PR). B - white-eyed (WP), pink eyed (PP) and black-eyed pupae (BP) and body pigmented pupae (BB). Arrows: mandibles.

Some mandible sections were subjected to histochemical analysis of mercury-bromophenol for protein detection and Periodic acid-Schiff (PAS) for neutral polysaccharide detection according to protocols from Pearse (1985) and Bancroft & Gamble (2007). Negative control for PAS test was performed by omitting periodic acid oxidation.

Results

The morphology of the intramandibular glands was characterized in adult workers, followed by monitoring of their development from the prepupae stage.

Adult

Mandibles of *P. verenae* adult workers had two classes of intramandibular glands, class I epidermal secretory cells and class III isolated glands. The class I secretory cells were characterized as columnar epidermal cells with well developed spherical nucleus and a homogeneously acidophilic cytoplasm (Fig. 2A). Class III isolated glands were scattered within the mandible cavity and showed well developed spherical nuclei and strongly basophilic cytoplasm with many vacuoles (Fig. 2B).

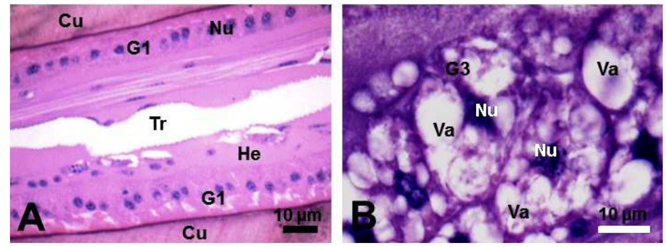

Fig. 2. Histological sections of the mandibles in adult workers of *Pachycondyla verenae*. A - Class I epidermal secretory cells (G1) characterized by cubic cells. Trachea (Tr). B - Class III unicellular glands of (G3) with vacuoles (Va) in the cytoplasm containing and spherical nucleus (Nu) with predominance of uncondensed chromatin. Cu: cuticle. He: hemocoel. Hematoxyline and eosin stained.

Histochemical analyses of both intramandibular gland types were positive for the presence of neutral polysaccharides and proteins.

Prepupae

In the mandibles of *P. verenae* prepupae, free cells were not found within the intramandibular cavity, which was filled with flocculent material. The epidermis was formed by flattened cells (Fig. 3), without features of glandular secretory cells.

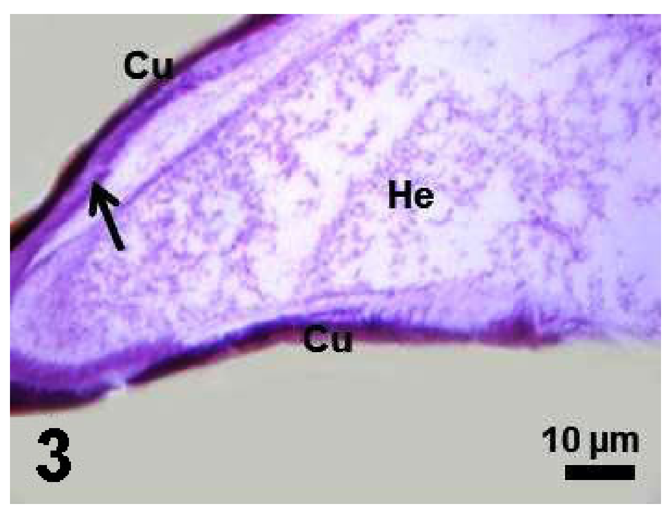

Fig. 3. Histological section of mandible of *Pachycondyla verenae* prepupae. A - flattened epidermis (arrow) and mandibular cavity, hemocoel (He) filled with flocculent material. Cu: cuticle. Hematoxyline and eosin stained.

White-eyed pupae

In white-eyed pupae of *P. verenae*, the mandible cavity was characterized by intense cell reorganization, with cells displaying irregular distribution and filamentous projections.

Table I - Histochemical tests in the intramandibular glands types I (G1) and III (G3) of *Pachycondyla verenae* (Formicidae: Ponerini) during different developmental stage of prepupae (PP), white-eyed pupae (WEP), pink-eyed pupae (PEP), black-eyed pupae (BEP), body pigmented pupae (BPP) and adult.

Histochemical test	Developmental stage											
	PP		WEP		PEP		BEP		BPP		Adult	
	G1	G3	G1	G3	G1	G3	G1	G3	G1	G3	G1	G3
Protein	ga	ga	ga	ga	ga	ga	+	+	+	+	+	+
Neutral polysaccharide	ga	ga	ga	ga	+	+	+	+	+	+	+	+

ga - gland absent, + positive reaction

Cell identification was based on the presence of small nucleus (Fig. 4A) as well as aggregation of protein rich rectangular cells (Fig. 4B). The single layered epidermal cells were cubic (Fig. 4A, 4C), although in some mandible regions they remained flattened. In addition to cells, the mandible cavity displayed an accumulation of flocculent material positive for proteins (Fig. 4C) and neutral polysaccharides (Fig. 4D).

Pink-eyed pupae

In pink-eyed pupae of *P. verenae*, the mandible cuticle was thicker than in previous pupae and mandibular teeth were present (Fig. 5A inset). The mandibular epidermal cells were similar to class I secretory cells found in adult workers, but with variations in size and shape. Specifically, some cells were spherical while others were columnar (Fig. 5A). The epidermis within the mandible was composed of columnar epithelium on one side and a pseudostratified-like epithelium on the opposed side (Fig. 5A). In addition, we found sensitive cell precursors (neuroblasts) of mandibular sensilla closely associated with the epidermis (Fig. 5B).

In the mandible cavity, precursors of class III gland cells were dispersed and had clear cytoplasm, making it difficult to identify the cell boundaries (Fig. 5C, 5D). The nuclei of these cells had uncondensed chromatin (Fig. 5B, 5C). In addition to cells dispersed in the mandible cavity, there were some chromatic bodies, which were small and strongly basophilic (Fig. 5A, 5B).

The basal and lateral regions of the class I epidermal cells were positive for neutral polysaccharides (Fig. 5E) and proteins (Fig. 5F).

Black-eyed pupae

In black-eyed pupae of *P. verenae*, the mandible was almost entirely lined by cubic class I epidermal cells (Fig. 6A). The class III gland cells had well developed nuclei and cytoplasm that was homogeneous, without vacuoles (Fig. 6B). Chromatic bodies were rare within the mandible cavity.

Histochemical analyses were positive for neutral polysaccharides (Fig. 6C) and proteins (Fig. 6D) in both class I and III gland cells.

Body pigmented pupae

Pupae of *P. verenae* with a pigmented body had a mandible epidermis with class I cubic gland cells and class III gland cells with vacuolated cytoplasm (Fig. 7).

The histochemical tests results in different intramandibular glands of different developmental stages of *P. verenae* are summarized in Table I.

Discussion

The morphology and histochemistry of intramandibular glands in *P. verenae* adult workers described here are similar to those of other Ponerini ants reported by Martins & Serrão (2011).

The occurrence of intramandibular glands in *P. verenae* with class I epidermal secretory cells and class III isolated glands was similar to that of adult ants (Schoeters & Billen, 1994, Billen & Espadaler, 2002; Grasso et al., 2004; Roux et al., 2010; Martins & Serrão, 2011).

Both classes I and III of intramandibular glands in *P. verenae* workers began to differentiate during the pink-eyed pupae stage. In bees, intramandibular glands also differentiate in the pupal stage (Cruz-Landim & Abdalla 2002). In addition, mandibular glands appear during the pupal development of the ant *P. obscuricornis* (Lommelen et al., 2003).
Intramandibular gland differentiation in *P. verenae* was not observed in prepupa. During that larval stage, ants, likely other holometabolous insects, accumulates food reserves and increases in size (Wheeler & Martinez, 1995), followed by metamorphosis, which consists of the complete structural reorganization of larval organs to adult organs (Wheeler & Buck, 1992; Rosell & Wheeler, 1995; Roma et al., 2006; 2009).

The white-eyed pupae of *P. veranae* also had no intramandibular glands, however, cell reorganization at this stage did occur and was likely due to proliferation and differentiation of epidermal cells. The white-eyed pupae of Hymenoptera is the earliest period of internal organs modifications, because it has yet larval organs and absence of those present in adults (Soares et al., 2004, Azevedo et al., 2008, Santos et al., 2009b; Cruz-Landim et al., 2011).

Intramandibular glands characterized as class I epidermal secretory cells or class III isolated glands can be definitively identified during the pink-eyed pupae stage of *P. verenae* in a manner similar to likely the labial, post-pharyngeal, hypopharyngeal, and mandibular glands that also differentiate in *Camponotus rufipes* pupae (Gama 1978).

In body pigmented pupae of *P. verenae*, both classes I and III of intramandibular glands are almost completely differentiated, similar to that reported from the salivary system of *C. rufipes,* which reaches maximum differentiation during the body pigmented pupae stage, despite the lack of secretory activity within the glands (Gama, 1978). The same may be occurring in the intramandibular glands of *P. verenae*, as the amount of gland cells in body pigmented pupae seems low compared to the adult ants.

The cellular processes that result in tissue and organ changes during metamorphosis include addition of new cells by cell division, cell loss due to programmed cell death, and cell migration (Cruz-Landim, 2009). In the present study, we are unable to detect some of these processes, but after the black-eyed pupae stage of *P. verenae,* intramandibular glands were similar to those found in adult workers, as is the case for other internal organs of Hymenoptera (Neves et al., 2002, 2003, Soares, 2004, Azevedo et al., 2008; Santos et al., 2009b).

The presence of proteins and neutral polysaccharides in intramandibular glands from white-eyed pupae of *P. verenae* suggests that during this developmental stage, there is a high level of cellular activity derived from the metabolic cell machinery. In the leaf-cutter ant, *Atta sexdens rubropilosa,* class III secretory cells contain carbohydrate and protein releasing glucoconjugate compounds (Amaral & Caetano, 2006).

In conclusion, class I epidermal secretory cells and class III isolated cells of the intramandibular glands of *P. verenae* workers differentiate during pupation, with onset occurring in pink-eyed pupae, and completion occurring in black-eyed pupae.

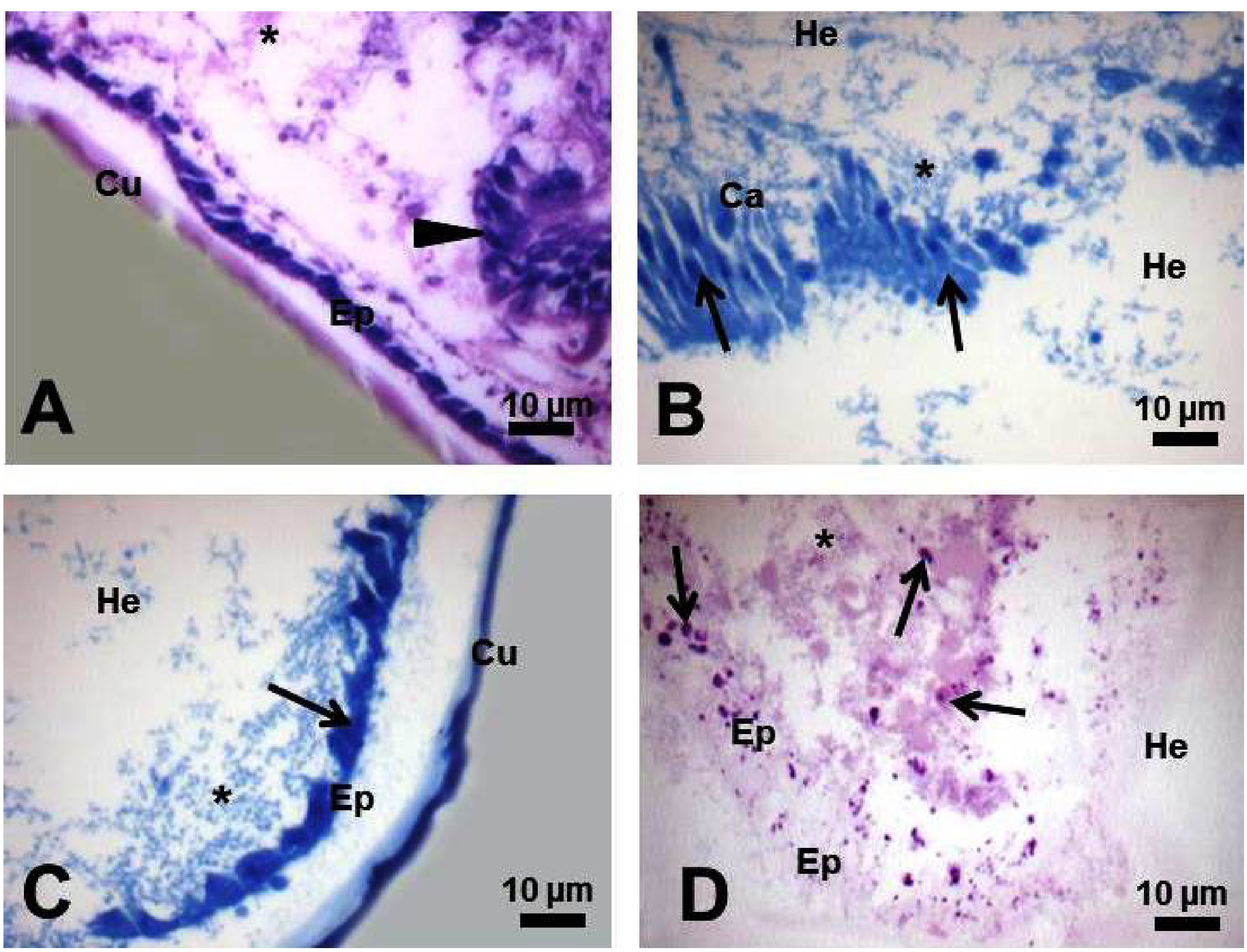

Fig. 4. Histological sections of mandible of *Pachycondyla verenae* white-eyed pupae. A- Epidermis (EP) with flattened cells and irregular cells in the mandible cavity with small nucleus (arrowheads) and flocculent material (*). Hematoxyline and eosin stained. B - Cell aggregate (Ca) and flocculent material (*) into mandible cavity positive (arrows) for protein. Mercury-bromophenol blue stained. C - epidermis (Ep) with cubic cells and flocculent material (*), both positive (arrows) for proteins. Mercury-bromophenol blue stained. D - flocculent material (*) and epidermis (Ep) positive for polysaccharides (arrows). Periodic acid- Schiff stained. He: hemocoel. Cu: cuticle.

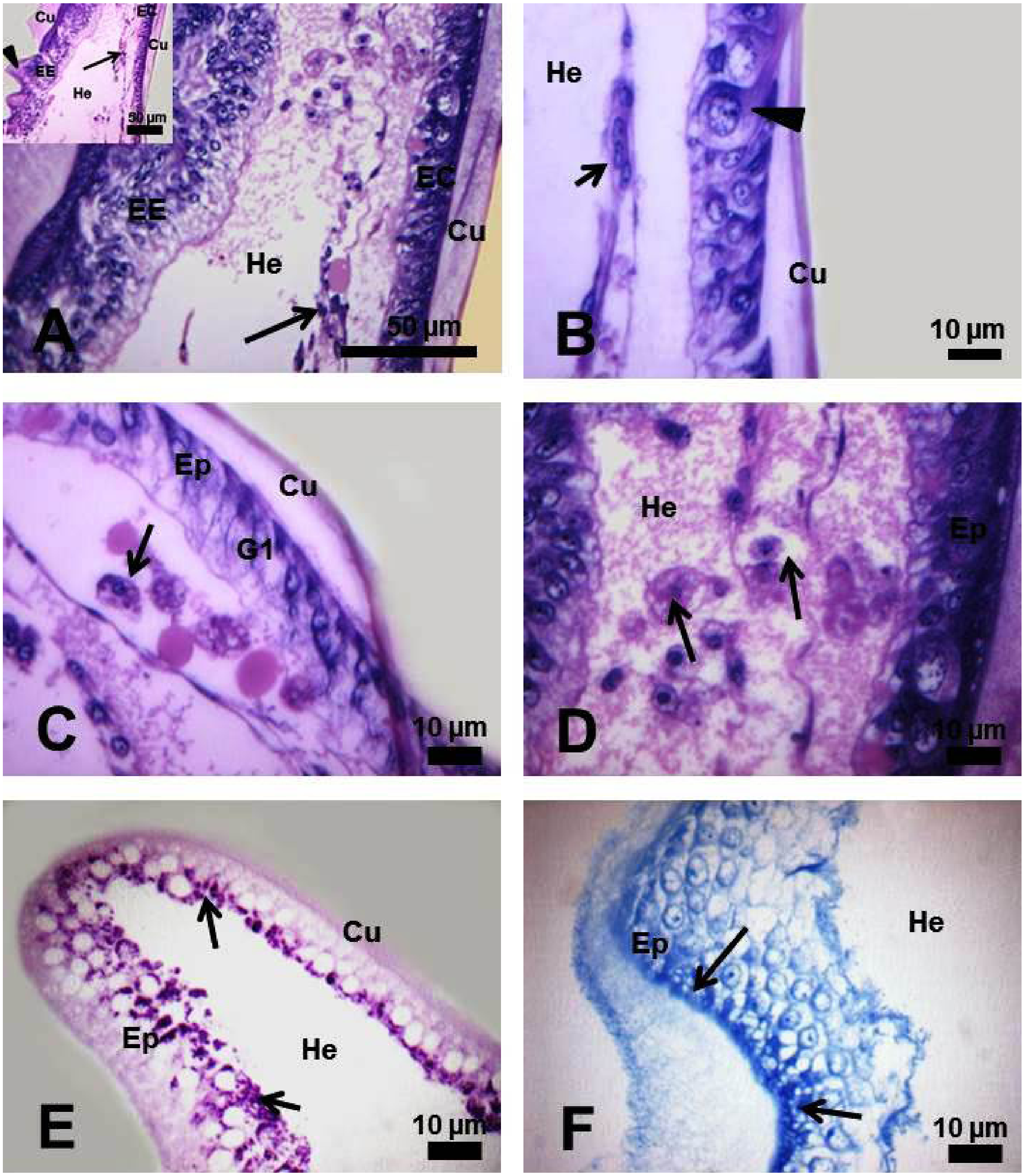

Fig. 5. Histological sections of the mandible of *Pachycondyla verenae* pink- eyed pupae. A - columnar (EC) and pseudostratified-like (EE) epidermis Note chromatic bodies (arrow) into mandible cavity Insert - general view of the mandible showing teeth (arrowhead). Hematoxyline and eosin stained. B - Neuroblast (arrowhead) in the epidermis and chromatic bodies (arrow) into mandible cavity (He). Hematoxyline and eosin stained. C - Class I epidermal gland showing columnar cells (GI). Hematoxyline and eosin stained. D - Class III precursor cells (arrows) with light cytoplasm into mandible cavity. Hematoxyline and eosin stained. E - positive reaction for polysaccharides (arrows) in the class I secretory cells. Periodic acid- Schiff stained. F - positive reaction for proteins (arrows) in class I secretory cells. Mercury-bromophenol blue stained. Cu: cuticle. Ep: epidermis. He: hemocoel.

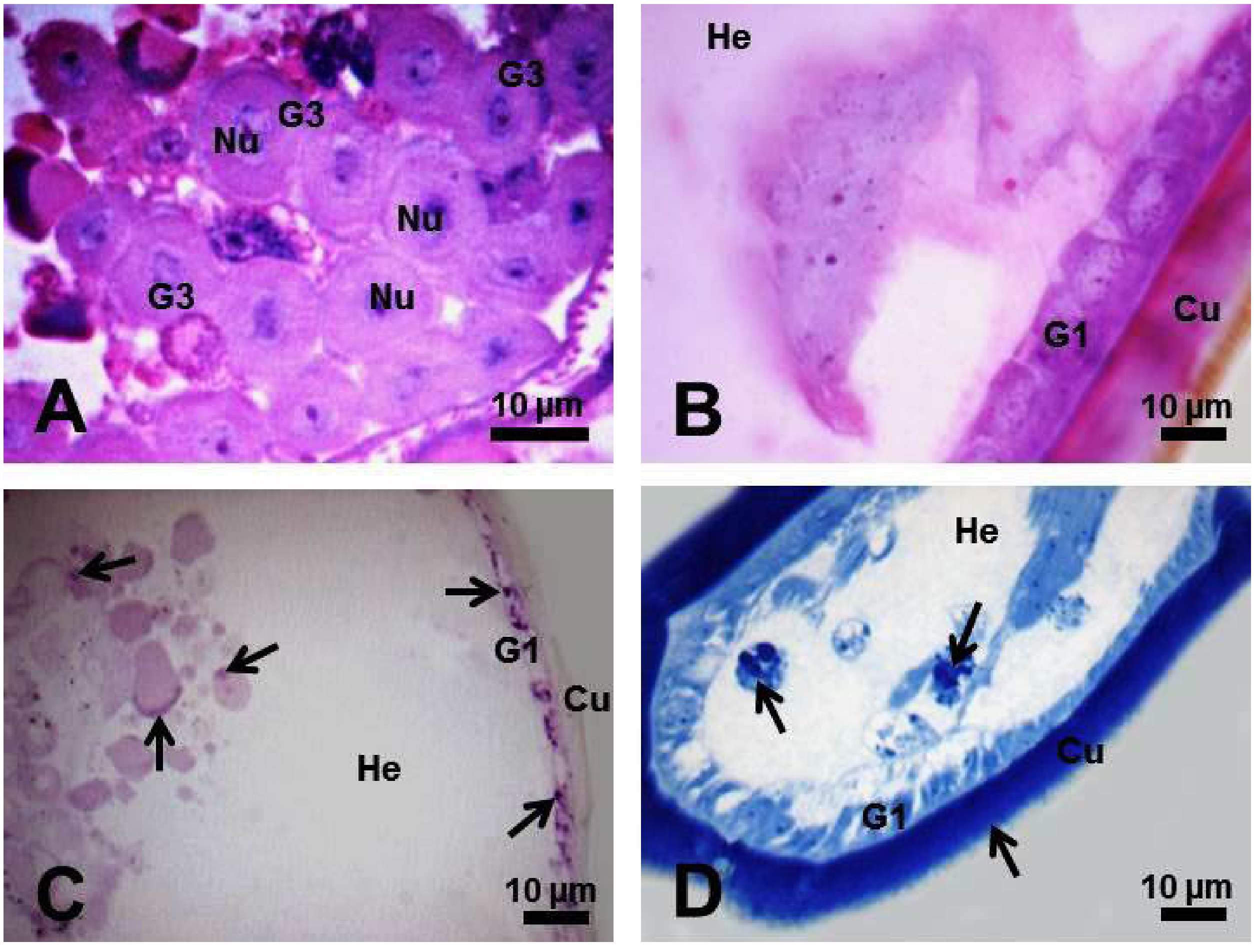

Fig. 6. Histological sections of the mandibles of *Pachycondyla verenae* black-eyed pupae. A - Class III unicellular glands of (GIII) showing well developed nucleus (Nu) and homogeneous acidophilic cytoplasm. Hematoxyline and eosin stained. B - Class I epidermal secretory cells (GI) showing nuclei with predominance of uncondensed chromatin. Hematoxyline and eosin stained. C - Positive reaction for polysaccharides (arrows) in the class I epidermal secretory cells (G1) and class III glands (G3). Periodic acid- Schiff stained. D - Positive reaction for proteins in the class I epidermal secretory cells (G1) and class III glands (G3). Mercury-bromophenol blue stained. Ep: epithelium. He: hemocoel. Cu: cuticle.

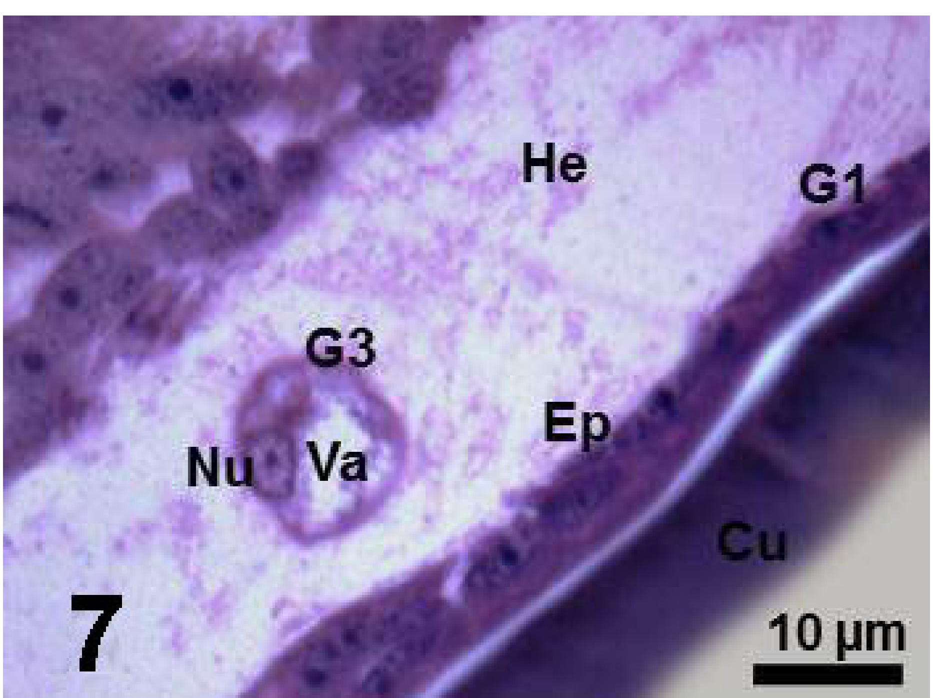

Fig. 7. Histological section of the mandible of body pigmented pupa of *Pachycondyla verenae*. A - well developed class I epidermal cells (G1) and Class III unicellular glands of (G3) with cytoplasm vacuoles (Va). Ep: epithelium. Hematoxyline and eosin stained. He: hemocoel. Cu: cuticle. Nu: nuclei.

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