Morphology of the Head Salivary and Intramandibular Glands of the Stingless Bee *Plebeia emerina* (Hymenoptera: Meliponini) Workers Associated with Propolis

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ABSTRACT *Plebeia emerina* (Friese) (Hymenoptera: Meliponini), like other stingless bees, collect large amounts of vegetal resin and store these materials, called propolis, in individualized clusters inside the nest that remain in a viscous state. The development of intramandibular and head salivary glands in *P. emerina* workers was studied in different life stages, aiming to relate gland functionality with the age in which they work at propolis maceration, biting the propolis clusters with the mandibles. The morphology of intramandibular and head salivary glands from newly emerged, 20–30 d old, and forager bees was analyzed. The greatest size of the head salivary glands occurred in 20–30-d-old worker bees, and the ultrastructure of this gland showed the presence of rough endoplasmic reticulum and lipid droplets. The intramandibular glands were of two types: glandular units (class 3 glands), present throughout the worker bee life span, and the secretory epithelium (class 1 glands), which hypertrophies in 20–30-d-old and forager bees. The development of the head salivary glands and the mandibular epithelium suggest that their products are added to the propolis clusters, supporting the hypothesis that they may serve in maintaining its viscous state.

KEY WORDS behavior, gland plasticity, life stages, propolis, stingless bees

Workers of stingless bees perform different tasks during their life span, which in some aspects can be adjusted to colony needs (Hebling et al. 1964, Simões and Bego 1991, Van Bethem et al. 1995). Worker capacity to perform different tasks may be associated with both the age of the bee and the physiological status of the exocrine glands (Costa-Leonardo and Cruz-Landim 1977). In spite of the many studies about exocrine glands in bees, studies on age polymorphism of these glands associated with worker tasks are sparse (Katzav-Gozansky et al. 2001; Deseyn and Billen 2004, 2005).

Exocrine glands in insects have an epidermal origin and can be classified into different types according their secretion-releasing apparatus. With class I glands, the columnar epidermis releases its secretion through the body cuticle, using the pore canals and resulting in cuticle deposition. Class III glands are those in which the cellular conducting canals open into pores in the cuticle (Cruz-Landim 1967; Noirot and Quennedey 1974, 1991; Costa-Leonardo and Cruz-Landim 1977; Salles and Cruz-Landim 1998; Cruz-Landim and Reginato 1999; Azevedo et al. 2007). The head salivary and intramandibular glands comprise the salivary system of bees (Cruz-Landim 1967, Michener 1974).

Head salivary glands are found in some species of Apinae and have multicellular alveoli with a thin cuticle lining a narrow lumen (Cruz-Landim 1967, Costa-Leonardo and Cruz-Landim 1977, Cavasin-Oliveira and Cruz-Landim 1998). These glands release an oily substance that probably aids worker bees as they work with wax during nest building (Heselhaus 1922), or in lubricating their mouthparts (Simpson 1960), or as trail pheromones (Jarau et al. 2004).

The stingless bees *Tetragonisca angustula* (Latreille) and *Plebeia* spp. use large amounts of propolis, storing a viscous cluster of propolis inside the nest (Nogueira-Neto 1997). This plastering resin can be used for nest defense when placed on the body of an invader (Kerr and Lello 1962). *Plebeia emerina* (Friese) workers, between 27 and 33 d old are frequently found macerating propolis onto the storage clusters (Santos 2007).

The addition of a glandular secretion from the workers' glands to the propolis cluster is thought to maintain its viscous state (Nogueira-Neto 1997). However, the exocrine glands responsible for the production of these substances are unknown. We analyzed the gross structure and ultrastructure of the head salivary and intramandibular glands of *P. emerina* workers of dif-

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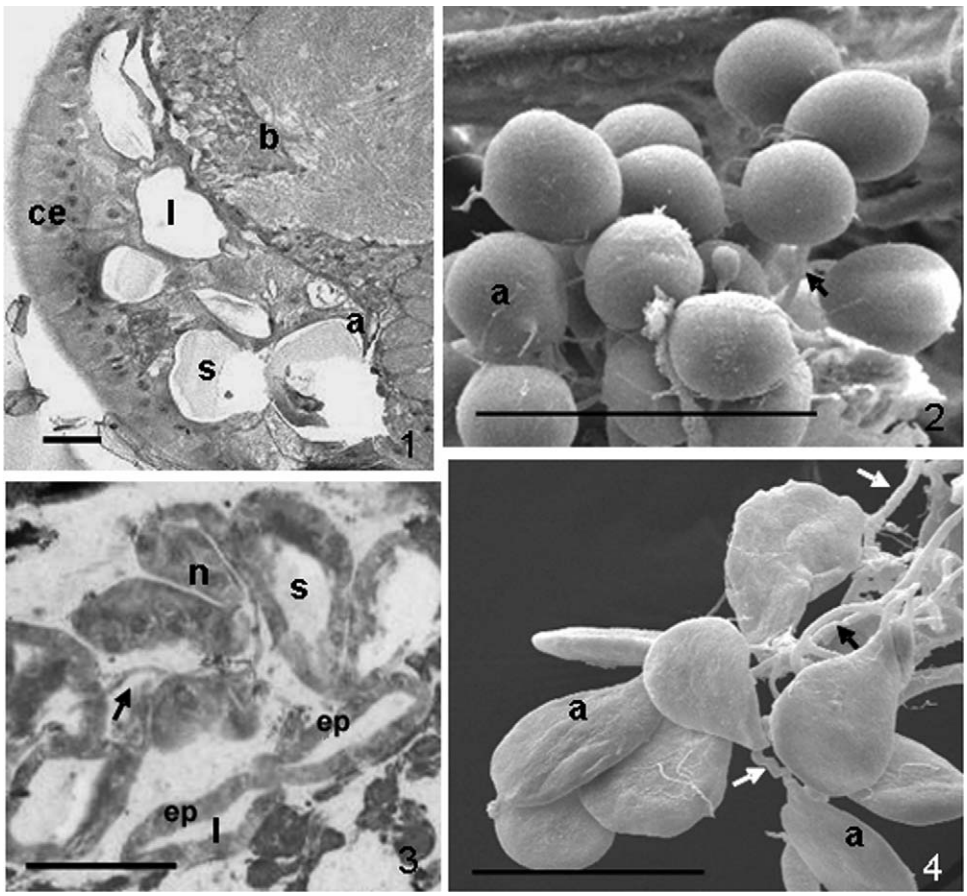


Figs. 1–4 Head salivary glands of *P. emerina* workers. 1. Histological section of the head showing enlarged alveoli (a) with flattened epithelium of the salivary gland in workers 20–30 d old. Bar = 50 μm. 2. Scanning electron micrograph of the spherical alveoli (a) in workers 20–30 d old. Bar = 100 μm. 3. Histological section of the gland alveolus with narrow lumen (l) and cuboidal epithelium (ep) in forager worker. Bar = 50 μm. 4. Scanning electron micrograph of forager worker showing elongated and flattened alveoli (a). Bar = 100 μm. b, brain; n, nucleus; ce, compound eye; s, gland content; arrows, excretory duct.

ferent ages to test the hypothesis that these glands may play a role in propolis maceration.

Materials and Methods

Animals. Workers of *P. emerina* were obtained from four colonies on the Campus Central Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, from October to December 2004 and 2005 (30° 03′38.64″ S, 051° 10′33.18″ W). Newly emerged, 20–30 d old bees (collected inside the nests), as well as forager workers (returning to the nest) were analyzed. The age of the bees was estimated by observing their thoracic color, because the cuticle of aging bees is darker (Fagundes et al. 2006).

Light Microscopy. Five workers of *P. emerina* from each age class were cryoanaesthetized for ≈1 min at −20°C, rapidly decapitated, and the heads were transferred to 4% aqueous paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.3. The specimens were dehydrated in a graded ethanol series and embedded in historesin (Leica, Heidelberg, Germany). Sections (4 μm in thickness) were stained with methylene blue and basic fuchsin.

Scanning Electron Microscopy. Mandibles of workers were removed and transferred to 5% potassium

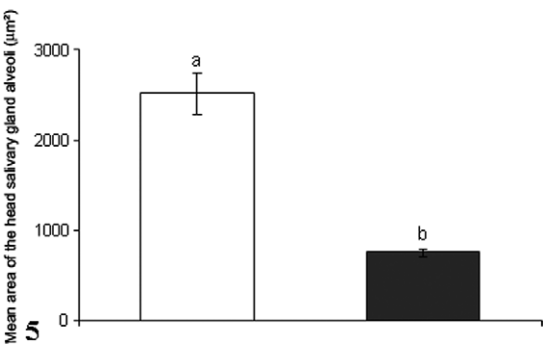
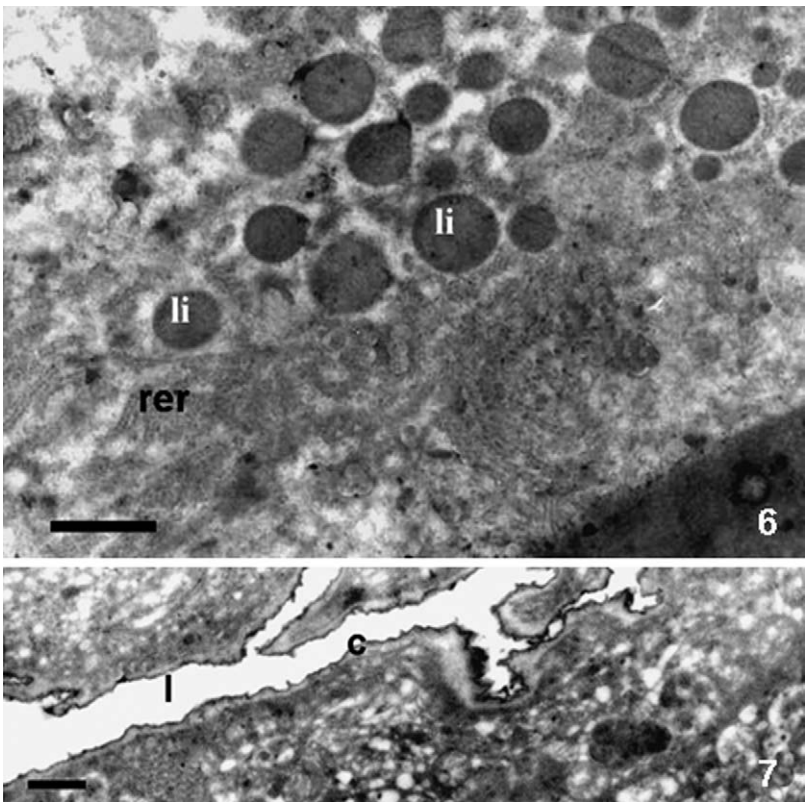


Fig. 5. Mean alveolar area of the head salivary gland in 20–30-d-old (white bar) and forager (black bar) workers of *P. emerina*. Columns with different letters differ by Student's *t*-test ($P < 0.05$). Bars indicate standard deviations.



Figs. 6–7 Transmission electron micrograph of the head salivary gland of a *P. emerina* worker. 6. Rough endoplasmic reticulum (rer) and lipid-like droplets (li) in the cell cytoplasm of 20–30-d-old workers. 7. Narrow and folded lumen (l) lined by a thin cuticle (c) in a forager worker. Bars = 1 μm.

hydroxide for 24 h, carbon covered (30 nm), and analyzed in a XL30 scanning electron microscope (SEM) (Philips, Eindhoven, Netherlands).

To study the ultramorphology of head salivary glands, five workers per age class (newly emerged, 20–30-d-old bees, and forager workers) were cryoanaesthetized, and the glands were dissected and transferred to 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7,3. The specimens were dehydrated in a graded series of ethanol and dried in a critical point dryer. The specimens were carbon coated (30 nm) and analyzed in the same SEM.

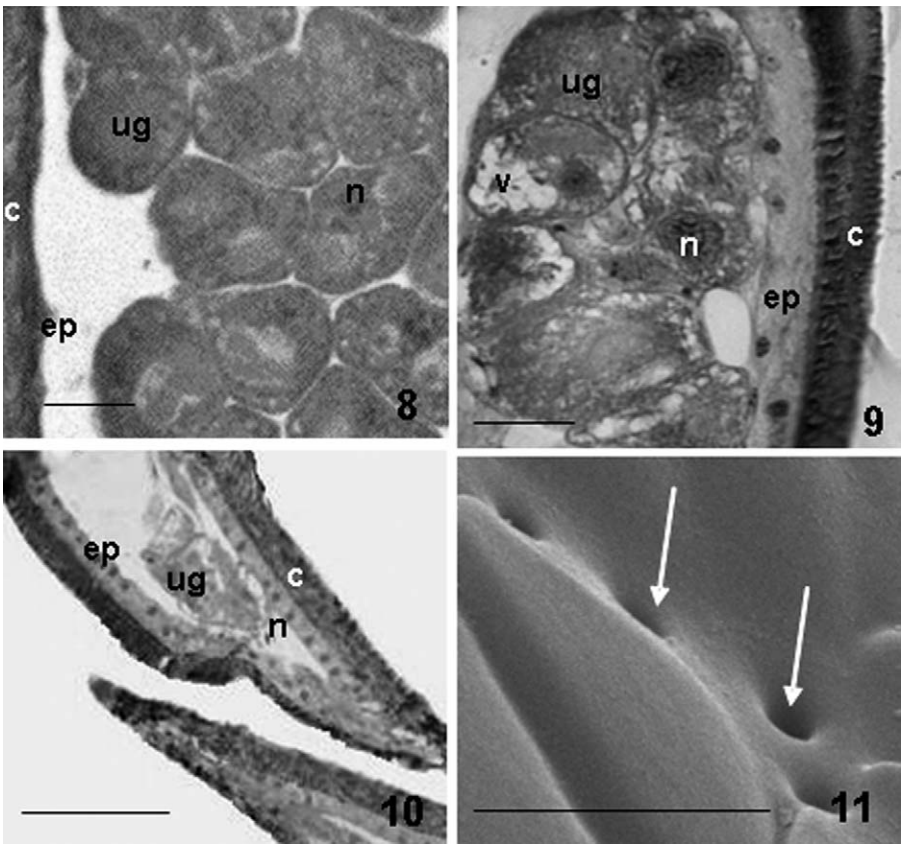
Transmission Electron Microscopy. The heads of five workers per age class were transferred to 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for at least 2 h. Both mandibles and the head salivary glands were dissected separately, postfixed in 1% osmium tetroxide in the same buffer, and embedded in Epon-Araldite. Ultrathin sections (100 nm in thickness) were stained with 1% uranyl acetate and lead citrate and analyzed in an EM 109 transmission electron microscope (Carl Zeiss, Jena, Germany).

Morphometrics and Statistical Analysis. Alveolar areas of both head salivary glands and secretory cells of class 3 intramandibular glands, as well as the height of the secretory epithelium of the intramandibular glands, were measured directly from histological sections with the aid of the software Image Pro-Plus 4.0 (Media Cybernetics, Silver Spring, MD). Alveoli from five sections per gland in five different workers per age were measured. The data from head salivary glands and secretory epithelium height of intramandibular glands from 20 to 30 d old workers and foragers were submitted to Student's t test at the 5% significance level. The data on areas of secretory cells of class 3 intramandibular glands in the three studied ages were not homogeneous nor normally distributed; therefore, they were submitted to the nonparametric Kruskal–Wallis test at the 5% significance level.

Results

In newly emerged workers of *P. emerina* head salivary glands were poorly developed showing almost vestigial alveoli, whereas 20–30-d-old workers and foragers had well developed glandular alveoli. In 20–30-d-old workers, alveoli were spherical with a flattened epithelial lining and an enlarged lumen filled with secretion (Figs. 1 and 2). In forager bees, the alveoli of head salivary glands were elongated with cuboidal epithelium and a narrow lumen (Figs. 3 and 4).

The alveolar area in 20–30-d-old workers was higher than in foragers ($t = 3.846$, $P < 0.01$) (Fig. 5). The secretory cells of the alveoli in 20–30-d-old workers



Figs. 8–11 Intramandibular glands of *P. emerina* workers. 8. Histological section of the mandible of newly emerged worker showing a flattened epithelium (ep) and unicellular glands (ug). Bar = 20 μm. 9. Histological section of the mandible of 20–30-d-old workers showing a hyperthrophied epithelium (ep) and unicellular glands (ug) with some vacuoles (v). Bar = 20 μm. 10. Histological section of the mandible in forager worker. Note the epithelium (ep) and unicellular glands (ug). Bar = 50 μm. 11. Scanning electron micrograph of the mandible of forager worker showing pores (arrows). Bar = 5 μm. c, cuticle; n, nucleus.

had a well-developed rough endoplasmic reticulum (RER) and some lipidlike droplets (Fig. 6). In forager workers, the cuticle lining the gland lumen was homogeneous with many folds (Fig. 7). Subcuticular space was narrow and the cell nucleus has condensed chromatin.

In all *P. emerina* workers, the intramandibular glands were of two types: a secretory epithelium and

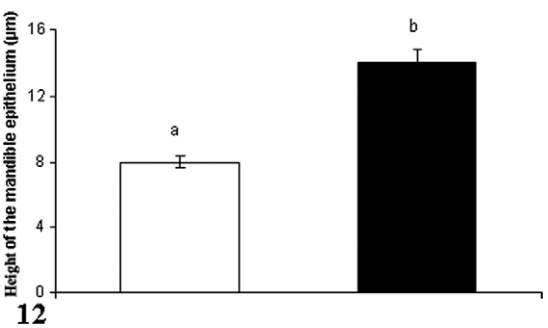


Fig. 12. Mean height of the mandible epithelium in 20–30-d-old (white bar) and forager (black bar) workers of *P. emerina*. Columns with different letters differ by Student's *t*-test ($P < 0.05$). Bars indicate SDs.

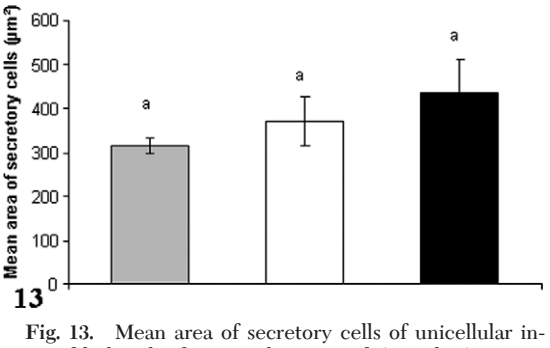
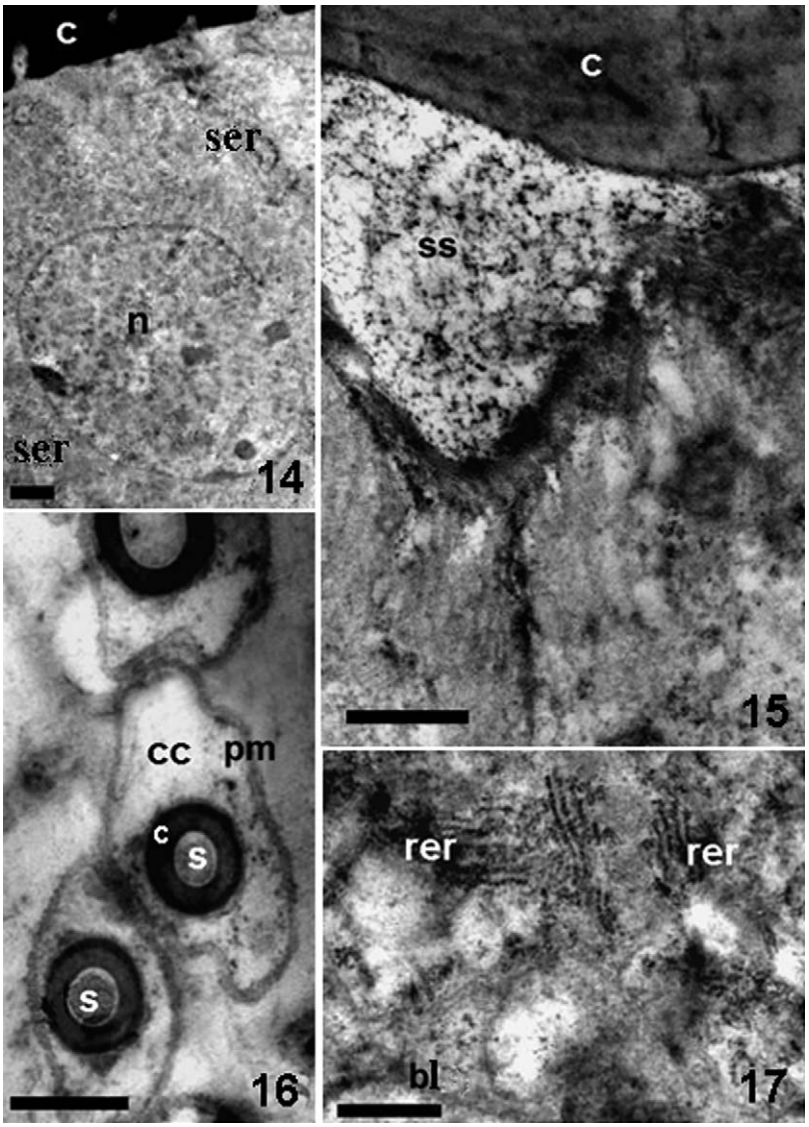


Fig. 13. Mean area of secretory cells of unicellular intramandibular glands in newly emerged (gray bar), 20–30-d-old (white bar), and forager (black bar) workers of *P. emerina*. Columns with the same letter do not differ by the Kruskal–Wallis test ($P < 0.05$). Bars indicate SDs.



Figs. 14–17 Transmission electron micrographs of the intramandibular glands of *P. emerina* workers. 14. Mandibular epithelium in a newly emerged worker showing the nucleus (n) and smooth endoplasmic reticulum (ser). Bar = 1 μm. 15. Cell apex of secretory epithelium in a 20–30-d-old worker showing the subcuticular space with electron dense content (ss). Bar = 0.5 μm. 16. The conducting canals (cc) of unicellular gland showing secretion (s) into the cuticle-lined lumen (c). pm, plasma membrane. Bar = 0.5 μm. 17. Basal cell region of the unicellular gland in 20–30-d-old workers showing rough endoplasmic reticulum (rer) and a thin basal lamina (bl). Bar = 0.5 μm.

unicellular glands (Figs. 8–11). The secretory epithelium was a class 1 gland and was poorly developed in newly emerged workers (Fig. 8) and was hypertrophied in 20–30-d-old and forager workers being most highly developed in forager workers ($t = 6.797$, $P < 0.001$) (Figs. 9, 10, and 12). In newly emerged workers the flattened cells of the mandibular epithelium were rich in smooth endoplasmic reticulum (SER) (Fig. 14), whereas in 20–30-d-old and forager workers the tall epithelium possessed a subcuticular space filled with electron-dense material (Fig. 15). These cells had well-developed RER (Fig. 17), a central nucleus with some nucleoli, and a thin basal lamina.

In *P. emerina* the unicellular glands were designated as class 3 intramandibular glands, and were found in all age classes without significant differences in degree of development (Kruskal–Wallis = 3.725, $P = 0.155$) (Fig. 13). These gland cells had an acidophilic cytoplasm with many granules in newly-emerged workers (Fig. 8), whereas in 20–30-d-old and forager workers, they were basophilic with many vacuoles, suggesting a constitutive release of their content (Fig. 16).

The mandibular surface of *P. emerina* has some clusters of pores at the opening of the conducting canal of the unicellular intramandibular glands (Fig. 11).

Discussion

In the honey bee, *Apis mellifera* L., the head salivary gland alveoli are large in forager workers (Katzav-Gozansky et al. 2001), whereas in some stingless bees, larger alveoli were found in 15-d-old workers followed by a degenerative process (Cruz-Landim 1967, 1968; Salles and Cruz-Landim 1998). Our results showed there was an enlargement in alveoli size from newly emerged workers with the maximum size in forager workers, suggesting a similar result to that described for honey bees, which may be due to the propolis manipulation performed by foraging honey bees workers (Michener 1974) and in older *P. emerina.* However, the difference in gland size associated with workers' age among *P. emerina* and other stingless bees maybe because the bee species studied by Cruz-Landim (1967, 1968) and Salles and Cruz-Landim (1998) do not store viscous cluster of propolis in their nest, as does *P. emerina.* In those species, older bees do not work at propolis manipulation; accordingly, they do not need a well-developed intramandibular gland.

The occurrence of RER and lipid droplets in cells of the head salivary glands in 20–30-d old workers suggests that these cells play a role in protein and lipids synthesis. Propolis maceration by *P. emerina* workers occurs between 27 and 33 d of adult age and coincides with an alveolar lumen filled with secretion. The relationship between worker behavior and gland morphology in this stingless bee suggests that head salivary gland products may be added to propolis during maceration and may play a role in maintenance of the viscous state of the propolis clusters.

The occurrence of two types of intramandibular glands in *P. emerina* corroborates previous findings that the mandibular epidermis is hypertrophied in some stingless bees (Costa-Leonardo 1978). The occurrence of well developed endoplasmic reticulum, nuclei with nucleoli, and accumulation of secretion in the subcuticular space suggest high epithelial gland activity in these bees.

Unicellular glands in the mandible have been reported in many stingless bees species, but in *A. mellifera* they are lacking (Nedel 1960, Cruz-Landim and Reginato 2001). Nedel (1960) stated that an oily secretion of the unicellular intramandibular glands plays a role in mandible lubrication. Our findings of well-developed glands in *P. emerina* workers during their whole life span corroborates Nedel's hypothesis. However, a well-developed secretory epithelium in 20–30-d-old and forager workers suggests that this gland may play a role in propolis manipulation and resin collection, because these workers manipulate propolis.

Our results revealed polymorphism in head salivary and intramandibular glands in *P. emerina* workers, and a maximum development of the former and of the secretory intramandibular epithelium in 20–30-d-old and forager workers. These findings suggest that their products may be mixed into the propolis clusters and lubricate the mouth parts, corroborating the hypothesis that these glands play a role in propolis manipulation and in maintenance of the viscous state of that stored resource.

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References Cited

Azevedo, D. O., E. W. Teixeira, M.L.T. Alves, A.C.C. Moreti, B. Blochtein, J. C. Zanuncio, and J. E. Serrão. 2007. Comparative analyses of the abdominal tergal glands in *Apis mellifera* (Hymenoptera: Apidae) queens. Anim. Biol. 57: 329–338.

Cavasin-Oliveira, G. M., and C. Cruz-Landim. 1998. Ultrastructure of Apoidea (Hymenoptera, Anthophoridae) salivary glands. I. Alveolar glands. Rev. Bras. Entomol. 42: 1–6.

Costa-Leonardo, A. M. 1978. Glândulas intramandibulares em abelhas sociais. Ciênc. Cult. 30: 835–838.

Costa-Leonardo, A.M.F., and C. Cruz-Landim. 1977. Estudo comparativo das glândulas do sistema salivar dos Apidae sociais (Hymenoptera). Rev. Bras. Biol. 37: 649–663.

Cruz-Landim, C. 1967. Estudo comparativo de algumas glândulas das abelhas (Hymenoptera, Apoidea). Arq. Zool. São Paulo 15: 177–290.

Cruz-Landim, C. 1968. Histoquímica e ultra-estrutura das glândulas salivares das abelhas (Hymenoptera, Apoidea). Arq. Zool. São Paulo 17: 113–166.

Cruz-Landim, C., and R. D. Reginato. 1999. Preliminary report on the presence of tegumental glands in the thorax of Meliponinae bees (Hymenoptera, Apidae). Rev. Bras. Biol. 59: 167–172.

Cruz-Landim, C., and R. D. Reginato. 2001. Exocrine glands of *Schwarziana quadripunctata* (Hymenoptera, Apinae, Meliponini). Braz. J. Biol. 61: 497–505.

Deseyn, J., and J. Billen. 2004. Age dependent morphology and ultrastructure of the thoracic labial gland of *Apis mellifera carnica* Pollm. Workers (Hymenoptera, Apidae). Belg. J. Zool. 134: 55–59.

Deseyn, J., and J. Billen. 2005. Age-dependent morphology and ultrastructure of hypoharyngeal gland of *Apis mellifera* (Hymenoptera, Apidae). Apidologie 36: 49–57.

Fagundes, I. B., L.A.O. Campos, and J. E. Serrão. 2006. Tergite pigmentation indicates hypopharyngeal gland developmental degree in *Melipona quadrifasciata* (Hymenoptera: Apidae: Meliponini). Sociobiology 48: 51–62.

Hebling, N. J., W. E. Kerr, and F. S. Kerr. 1964. Divisão de trabalho entre operárias de *Trigona* (*Scaptotrigona*) *xanthotricha* Moure. Papéis Av. Zool. 16: 115–127.

Heselhaus, F. 1922. Die Hautdrüsen der Apiden und Verwandter Formen. Zool. Jahrb. Abt. Anat. 43: 363–464.

Jarau, S., M. Hrncir, R. Zucchi, and F. G. Barth. 2004. A stingless bee uses labial gland secretions for scent trail communication (*Trigona recursa* Smith 1863). J. Comp. Physiol. 190A: 233–239.

Katzav-Gozansky, T., V. Soroker, A. Ionescu, G. E. Robinson, and A. Hefetz. 2001. Task-related chemical analysis of labial gland volatile secretion in worker honeybees (*Apis mellifera ligustica*). J. Chem. Ecol. 27: 919–926.

Kerr, W. E., and E. Lello. 1962. Sting glands in stingless bees – A vestigial character. J. N Y Entomol. Soc. 70: 190–214.

Michener, C. D. 1974. The social behavior of the bees. Belknap Press of Harvard University Press, Cambridge, MA.

Nedel, O. J. 1960. Morphologie und physiologie der mandibeldrüse einiger Bienen-Arten (Apidae). Z. Morphol. Tiere 49: 139–183.

Nogueira-Neto, P. 1997. Vida e criação de abelhas indígenas sem ferrão. Ed. Nogueirapis, São Paulo, Brazil.

Noirot, C., and A. Quennedey. 1974. Fine structure of insect epidermal glands. Annu. Rev. Entomol. 19: 61–80.

Noirot, C., and A. Quennedey. 1991. Glands, gland cell, glandular units: some comments on terminology and classification. Ann. Soc. Entomol. Fr. 27: 123–128.

Santos, C. G. 2007. Aspectos comportamentais e morfológicos de *Plebeia emerina* (Friese, 1900) (Hymenoptera, Apidae, Meliponini) relacionados à própolis. Ph.D. dissertation, Faculdade de Biociências da PUCRS, Porto Alegre, Brazil.

Salles, H. C., and C. Cruz-Landim. 1998. Levantamento das glândulas exócrinas presentes em *Camargoia nordestina* Moure, 1989 (Hymenoptera, Apidae, Meliponinae). Rev. Bras. Entomol. 41: 297–302.

Simões, D., and L. R. Bego. 1991. Division of labor, average life span and life table in *Nannotrigona* (*Scaptotrigona*) *postica* Latreille (Hymenoptera, Apidae, Meliponinae). Naturalia 16: 81–97.

Simpson, J. 1960. The functions of the salivary glands in *Apis mellifera.* J. Insect Physiol. 4: 107–121.

Van Bethem, F.D.J., V. L. Imperatriz-Fonseca, and H. W. Velthuis. 1995. Biology of the stingless bee *Plebeia remota* (Holmberg). Observation and evolutionary implications. Insectes Soc. 42: 71–87.

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