Tegumentar glands associated to foveae in the second metasomal tergum of Panurgillus Moure (Apoidea, Andrenidae, Panurginae)

Kelli dos Santos Ramos^{1,2}, Camila Gonçalves dos Santos², Clemens Schlindwein³ & Betina Blochtein²

¹Bolsista CNPq/PIBIC. E-mail: kellisbio@yahoo.com.br

ABSTRACT. Panurginae have a pair of cuticular depressions in the second metasomal tergum, recognized as lateral foveae of the T2. These structures have been used as systematic and taxonomic characters, although their functions are yet unknown. We aimed a morphological analysis at lateral foveae of three species of *Panurgillus* Moure, 1998: *P. vagabundus* (Cockerell, 1918), *P. reticulatus* Schlindwein & Moure, 1998 e *P. flavitarsis* Schlindwein & Moure, 1998. The study of the external morphology showed that the lateral foveae of the T2 are evident among females, but in males they are undistinguishable or absent. The surface of the foveae is micropunctuated in all species. The histological analysis has shown that the region of the lateral foveae of the T2, of female and male of the three species, presented tegumentar specializations. The inner part showed an evident secretory epithelium recognized as Class I gland. The height of this secretory epithelium was not uniform, although the cellular features are similar independent of sex. We have not found any previous information regarding the presence of glands related to abdominal foveae in Panurginae species.

KEYWORDS. Abdomen; gland; Panurgillus; T2 lateral foveae; tegumentar specialization.

Resumo. Glândulas tegumentares associadas às fóveas do segundo tergo metasomal de *Panurgillus* Moure (Apoidea, Andrenidae, Panurginae). Panurginae apresenta um par de depressões cuticulares no segundo tergo metasomal, denominadas fóveas laterais do T2. Essas estruturas têm sido usadas como caráter sistemático e taxonômico, entretanto suas funções ainda são desconhecidas. Visamos a análise morfológica das fóveas laterais de três espécies de *Panurgillus* Moure, 1998: *P. vagabundus* (Cockerell, 1918), *P. reticulatus* Schlindwein & Moure, 1998 e *P. flavitarsis* Schlindwein & Moure, 1998. O estudo da morfologia externa mostrou que as fóveas laterais do T2 são evidentes nas fêmeas, mas inconspícuas ou ausentes nos machos. A superfície das fóveas é micropontuada em todas espécies. A análise histológica da região das fóveas laterais do T2, de fêmeas e machos das três espécies, mostrou a presença de especializações tegumentares. Na parte interna observa-se um evidente epitélio secretor reconhecido como glândula da Classe I. A altura deste epitélio secretor não é uniforme embora as características celulares sejam similares independentemente do sexo. Não foi encontrada qualquer informação anterior a respeito da presença de glândulas relacionadas às fóveas abdominais em espécies de Panurginae.

PALAVRAS-CHAVE. Abdômen; glândula; Panurgillus; fóvea lateral do T2; especialização tegumentar.

The Panurginae are small, generally solitary and oligolectic bees in the Asteraceae, Cactaceae, Iridaceae, Malvaceae, Oxalidaceae, Solanaceae and Verbenaceae flowers among others (Schlindwein 1995; Alves-dos-Santos 1999). The subfamily presents its highest diversity in the North and South America, are very scarce in the tropical region and absent in Australia and Tropical Asia (Michener 1979; 2000). In the southern region of Rio Grande do Sul, Panurginae is represented by 28 species (Schlindwein 1998). The genus *Panurgillus* Moure, 1998 is remarkable and constituted by 21 species, found from the Province of Salta (Argentina) up to the state of Rio de Janeiro (Brazil) (Schlindwein & Moure 1998, 1999).

In the adults of Panurginae is present a pair of cuticular depressions in the second metasomal tergum recognized as T2 lateral foveae. Ruz (1991) refers to the T2 foveae as one of the characters for identification of the subfamily. Moreover, these structures are used in the taxonomy and systematic of different genus, among them *Psaenythia* Gerstäcker, 1868,

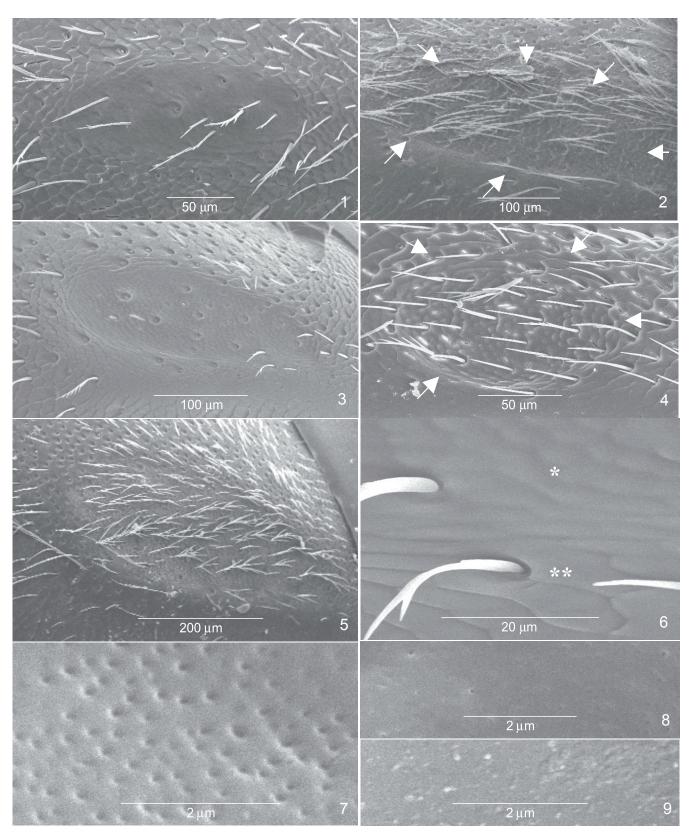
Anthrenoides Ducke, 1907, Protandrena Cockerell 1896, Neffapis Ruz, 1995, Cephalurgus Moure & Oliveira, 1962, Protomeliturga Ducke, 1912, Arhysosage Brèthes, 1922, Callonychium Brèthes, 1922, Acamptopoeum Cockrell, 1905, Spinoliella Ashmead, 1899, Calliopsis Smith, 1853 and Panurgillus Moure, 1998 (Moure & Oliveira 1962; Ruz 1991; Rozen & Ruz 1995; Schlindwein & Moure 1998, 1999; Michener 2000; Silveira et al. 2002).

Some specializations evidenced in the external morphology of bees are associated to differentiations in the corresponding epidermis. Solitary bees of the genus *Centris* Fabricius, 1804 (Apidae) and *Nomia* Latreille, 1804 (Halictidae) present changes in their legs, associated to tegumentar glands, whose secreting products influence their sexual behavior (Coville *et al.* 1986; Williams *et al.* 1984; Wcislo *et al.* 1992). Wittmann & Blochtein (1995) while studying the ventral portion of the dilated anterior basitarsus of *Megachile* (Megachilidae) species verified that males, during mating, release substances (pheromones) originating from basitarsus tegumentar glands

²Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul. Caixa postal 1429, 90619-900 Porto Alegre-RS, Brazil. E-mail: bblochtein@pucrs.br

³Departamento de Botânica, Universidade Federal de Pernambuco. Av. Prof. Moraes Rêgo s/n, 50670-901 Recife-PE, Brazil. E-mail: schlindw@ufpe.br

Ramos et al.



Figs. 1-9. T2 lateral foveae of *Panurgillus reticulatus*: 1, female; 2, male. *Idem P. vagabundus*: 3, female; 4, male. *Idem P. flavitarsis*: 5, female. Detail of the external surface of the foveae and adjacent area of *P. vagabundus*: 6, * sculpturation in the ** adjacent to foveae. *P. reticulatus*: 7, dense punctuation in foveae. *P. vagabundus*: 8, sparse punctuation in foveae; 9, adjacent to foveae without punctuations. (arrows) outline of T2 lateral foveae.

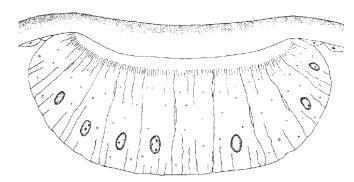


Fig. 10. Gland scheme associated to the T2 foveae region.

on the antennae of the females.

Tegumentar glands are derived from the epidermis and remain linked to it, as a final way of eliminating their products (Staurengo-da-Cunha *et al.* 1990). According to Noirot & Quennedey's (1974, 1991) classification, two patterns of glands are recognized among bees: Class I (secretory epithelium) and Class III (glandular units). In the Class I, the epithelium itself assumes the glandular function. The glands of Class III are formed by groups of secretory cells linked to the tegument by individual canals that perforate the cuticle to release their product.

SCHLINDWEIN & MOURE (1998) observed that among males and females of *Panurgillus vagabundus* (Cockerell, 1918) and *P. reticulatus* Schlindwein & Moure, 1998 and among females of *P. flavitarsis* Schlindwein & Moure, 1998 the T2 foveae are present. Until now, the T2 foveae were mentioned in the literature only under the taxonomic focus, without the comprehension of its function. Therefore, the objective of this work is to do a morphological study of the region of the T2 lateral foveae among males and females of three species of *Panurgillus*.

MATERIAL AND METHODS

Bee Collection. The specimens of *Panurgillus* vagabundus were collected in Porto Alegre (October/1996) and *P. reticulatus* and *P. flavitarsis* in the Centro de Pesquisas

e Conservação da Natureza Pró-Mata located in São Francisco de Paula (January and October/2001), RS, Brazil. The individuals of *P. flavitarsis* were obtained in *Ludwigia* Linnaeus (Onagraceae) flowers, whereas *P. reticulatus* and *P. vagabundus* in *Oxalis* Linnaeus (Oxalidaceae).

Scanning Electron Microscopy. For the analysis of the external morphology of the T2 foveae, the abdomen of each species was submersed in a detergent solution (Extran®) in a heated agitator (60-70°C) for 2 hours and after, it was washed in distilled water. The specimen was then dehydrated in crescent series of alcohol, transferred to acetone solution, sonicated for 10 minutes and air dried at 40°C for 24 hours. Abdomens were fixed in a stub, covered with carbon and gold and analyzed under a Scanning Electron Microscope Philips XL 30.

Light Microscopy. After the collection, the individuals were cold immobilized (4°C/5 minutes) and transferred to 4% Paraformaldehyde (buffered phosphate 0,1M pH 7,3). After this, the abdomen of males and females were microdissected to isolate the second metasomal tergum. The samples were dehydrated in a crescent series of alcohol up to 95%, embedded in Hydroxyethyl-methacrylate (Leica Historesin®) under vacuum (60min/0,9 Kp cm²) and the polymerization was conducted in an incubator at 40°C. Serial sections (4μm) were carried out in a microtome Leica-Jung RM 2145 with tungstenium blades. The sections were stained with basic methylene/fucsina, analyzed and photographed under a Axioskop Zeiss microscope. The cuticle and epithelium of 2 specimens of each sex (12 individuals) were sized with a milimetric ocular (Table I).

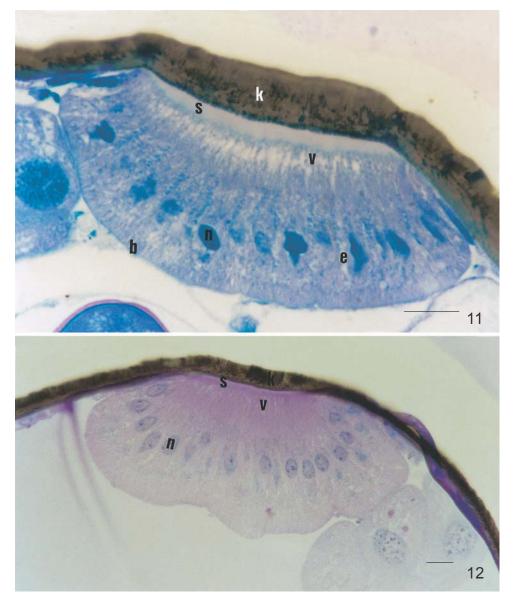
RESULTS AND DISCUSSION

Scanning Electron Microscopy. The T2 lateral foveae were different concerning their form and size among the species and also concerning the sex (Figs. 1-5). The dimensions of the T2 foveae obtained in this study were similar to the ones found by Schlindwein & Moure (1998) (Table I). Among the females, the T2 foveae are evident, whereas among the males they are inconspicuous or absent in *P. flavitarsis* (Table I).

Table I. Dimensions of structures related to lateral foveae of the T2 (FT2) among three species of *Panurgillus*. *Results found by Schlindwein & Moure, 1998. ++ conspicuous; + inconspicuous; - absent.

Species /	P. flavitarsis		P. vagabundus		P. reticulatus	
Sex	Male	Female	Male	Female	Male	Female
FT2	_	++	+	++	+	++
Length x width of T2 lateral foveae (mm)*	-	0.5×0.24	$0,14 \times 0,06$	$0,34 \times 0,1$	0.3×0.12	$0,12 \times 0,06$
Length x width of T2 lateral foveae (mm)	-	$0,36 \times 0,2$	0.13×0.07	$0,25 \times 0,1$	$0,24 \times 0,12$	$0,19 \times 0,08$
Thickness of cuticle in foveae (μm)	15,4	14,6	17,0	15,0	13,5	12,2
Thickness of adjacent cuticle to foveae (µm)	11,7	11,2	12,2	11,7	10,2	11,0
Height of epithelium infoveae (μm)	67,0	57,0	38,0	45,0	65,0	88,0

166 Ramos et al.



Figs. 11-12. Glandular epithelium associated to lateral foveae of the T2 of *Panurgillus reticulatus*: 11, male; 12, female. (b) basal labyrinth, (n) nucleus, (e) intercellular space, (v) microvilli (s) subcuticular space and (k) cuticle. Scale: 14 μm

The foveae in the examined specimens were covered by short, straight or subdivided hairs. These hairs were similar to those found in the tegument adjacent to the foveae but the surface of the foveae presents a lighter microsculpturation compared with the adjacent region (Fig. 6).

The ultrastructural characterization of the tegumentar surface of the T2 foveae and adjacent regions demonstrates that the punctuations differ concerning their presence and density (Figs.7-9). On the foveae of the studied species, the punctuations are similar to the ones found in adjacent regions, except in females of *P. vagabundus* (Fig. 9) and *P. reticulatus* in which they are absent outside the foveae. The punctuation in the surface of the foveae is dense (Fig. 7), except in males of *P. vagabundus*, in which it is sparse (Fig. 8).

Light Microscopy. The region of the T2 lateral foveae of males and females of *P. flavitarsis*, *P. reticulatus* and *P. vagabundus* has revealed tegumentar specializations. The inner surface correspondent to the foveae, presented an evident secretory epithelium (Fig. 10), which can be classified as being gland of Class I according Noirot & Quennedey (1991). This secretory epithelium is characterized by presenting cylindrical cells, a wide basal labyrinth, basal nucleus containing from one to three nucleoli and evident and widened intercellular spaces. Cytoplasmatic granules configure a gradient up to the apical region, where microvilli may be seen as well as the presence of a subcuticular space (Figs. 11-12).

Among the three species studied we found that the cuticle in the region of the T2 foveae is thicker than in the adjacent

areas. (Table I). The maximum height of the secretory epithelium, associated to the region of the T2 lateral foveae, varied from 38 to 67µm among males and from 45 to 88µm among females. *P. flavitarsis* was the only species in which males, although the foveae were absent, presented a maximum height of the secretory epithelium when compared to females. These variations in the height of the secretory epithelium may be related to inter and intra-specific physiological and/or ontogenetic differences. Although there are variations, the gland in the T2 foveae among the three examined species presents similar cell characteristics independent of sex.

STAURENGO-DA-CUNHA *et al.* (1990) stated that although the tegumentar glands are genetically determined, the state of development and the chemical nature of the products secreted may vary. Their activity is influenced by the environment and is adaptable to circumstances according to species or individual.

Petrúcio de Medeiros & Clemens Schlindwein (personal communication) when studying the mating behavior among *Protomeliturga turnerae* (Ducke 1907) (Andrenidae) observed that males would scratch the legs in the lateral region of the metasomal and immediately after this they would walk on the flower. Right after this, a female landed on the same flower and scratched its legs on the metasomal terga. Immediately after the same male landed on the female and they started copulating. This behavior might indicate the use of products that are secreted by glands which are associated to the T2 foveae present in this species.

The comprehension of functional roles of the T2 glands of *Panurgillus* depends on histochemical and behavioral studies. This is the first record of the presence of glands associated to abdominal foveae among Panurginae species.

Acknowledgements. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq. We thanks the histology team (K. Castro, A. Amaral, L. Lopes, S. Witter, R. Rebello) for colleting the bees, Birgit Harter-Marques for the identification of the bees and Maria Antonieta Lopes de Souza for kindly checking the English of this paper.

REFERENCES

ALVES-DOS-SANTOS, I. 1999. Abelhas e plantas melíferas da mata atlântica, restinga e dunas do litoral norte do estado do Rio Grande do Sul, Brasil. Revista Brasileira de Entomologia 3(4): 190-223.
COVILLE, R. E.; G. W. FRANKIE; S. L. BUCHMANN; S. B. VINSON & H. J.

- WILLIAMS. 1986. Nesting and male behavior of *Centris heithausi* (Hymenoptera; Anthophoridae) in Costa Rica with chemical analysis of the hindleg glands of males. **Journal of the Kansas Entomological Society 59**(2): 325-336.
- Michener, C. D. 1979. Biogeography of the bees. Annals of the Missouri Botanical Garden 66: 277-347.
- Michener, C. D. 2000. The Bees of the World. Baltimore, John Hopkins University Press, 913 p.
- Moure, J. S. & B. L. Oliveira. 1962. Novo gênero de Panurginae para América do Sul. **Boletim da Universidade Federal do Paraná: Zoologia 15**: 1-14.
- NOIROT, C. & A. QUENNEDEY. 1974. Fine structure of insect epidermal glands. Annual Review of Entomology 19: 61-80.
- NOIROT, C. & A. QUENNEDEY. 1991. Glands, gland cells, glandular units: some comments on terminology and classification. Annales de la Societé Entomoloque de France 2(27): 123-128.
- ROZEN, J. G. & L. Ruz. 1995. South American panurgine bees (Andrenidae; Panurginae), part II. Adults, immature stages, and biology of *Neffapis longilingua*, a new genus and species with an elongate glossa. American Museum Novitates 3136: 1-15.
- Ruz, L. 1991. Classification and phylogenetic relationships of the panurgine bees: the Calliopsini and Allies (Hymenoptera; Andrenidae). The University of Kansas Science Bulletin 54(7): 209-256.
- Schlindwein, C. 1995. Melittophilous plants, their pollen and flower visiting bees in Southern Brazil. 2.Cactaceae. **Biociências 3**(2): 35-71
- SCHLINDWEIN C. 1998. Frequent oligolecty characterizing a diverse beeplant community in a xerophytic bushland of subtropical Brazil. Studies on Neotropical Fauna and Environment 33: 46-59.
- SCHLINDWEIN, C. & J. S. MOURE. 1998. Panurgillus gênero novo de Panurginae, com a descrição de quatorze espécies do sul do Brasil (Hymenoptera; Andrenidae). Revista Brasileira de Zoologia 15(2): 397-493.
- SCHLINDWEIN, C. & J. S. MOURE. 1999. Espécie de *Panurgillus* Schlindwein & Moure (Hymenoptera; Andrenidae) depositados no Naturkunde Museum, Berlim. Revista Brasileira de Zoologia 16(1): 113-133.
- SILVEIRA, F. A.; G. A. R. Melo & E. A. B. Almeida. 2002. Abelhas Brasileiras: Sistemática e Identificação. Belo Horizonte, IDMAR, 253 p.
- Staurengo-da-Cunha, M. A.; C. Cruz-landim & L. A. O. Campos. 1990. Ocorrência e morfologia das glândulas tegumentares de algumas espécies de abelhas (Hymenoptera; Apoidea). **Naturalia 15**: 209-217.
- WILLIAMS, H. J.; S. B. VINSON; G. W. FRANKIE; R. E. COVILLE & G. W. IVIE. 1984. Morphology, chemical contents and possible function of the tibial gland of males of the Costa Rica solitary bees *Centris* nitida and *Centris trigonoides subtarsata* (Hymenoptera; Anthophoridae). Journal of the Kansas Entomological Society 57(1): 50-54.
- WSCILO, W. T.; R. L. MINCKLEY & H. C. SPANGLER. 1992. Pre-copulatory courtship behavior in a solitary bee, *Nomia triangulifera* Vachal (Hymenoptera; Halictidae). **Apidologie 23**: 431-442.
- WITTMANN, D. & B. BLOCHTEIN. 1995. Why males of leafcutter bees hold the females' antennae with their front legs during mating. Apidologie 26: 181-195.