1 **Structure and development of the metapleural gland in**

2 ***Technomyrmex vitiensis***

3 Pavel Pech¹ & Johan Billen²

4 ¹ Faculty of Science, University of Hradec Králové, Rokitanského 62, 500 03 Hradec
5 Králové, Czech Republic. E-mail: pavelpech1@centrum.cz (corresponding author)

6 ² KU Leuven, Zoological Institute, Naamsestraat 59, box 2466, B-3000 Leuven, Belgium.
7 E-mail: johan.billen@kuleuven.be

8 **Abstract**

9 We studied the internal and external appearance of the metapleural gland in the 5 castes
10 of *Technomyrmex vitiensis*. All castes do possess the gland, but with a clear gradient from
11 queens (most developed gland), via intercastes, workers and wingless males to winged
12 males (least developed). Also the size and the shape of the atrium differs among castes
13 along the same gradient. The presence of a more developed gland in wingless males than
14 in winged males may be linked with the much longer longevity of the former. The number
15 of secretory cells in *T. vitiensis* is the lowest among ants known so far. The functional
16 meaning of this is not yet known, and calls for a functional study of the gland's secretion.

17 **Introduction**

18 Among the various social insect groups, ants are known for having the largest number of
19 exocrine glands, with 84 different glands distinguished so far (Billen and Šobotník 2015).
20 In this impressive exocrine equipment, the metapleural gland is of special importance, as it
21 represents a neoformation that is only found in the Formicidae (Brown 1968). The gland is
22 present in the majority of species, although it is absent in some social parasites and

23 arboreal ants, while it is also often absent in males (Brown 1968; Hölldobler and Engel-
24 Siegel 1984). The metapleural gland is a paired structure situated in the posterior region of
25 the thorax. Each gland consists of secretory cells, each cell opening into a sac-like atrium
26 through an accompanying duct cell. The metapleural gland secretion is believed to have
27 an antimicrobial and fungicidal effect and should substantially contribute to ant immunity
28 (Maschwitz et al. 1970; Maschwitz 1974; Beattie et al. 1986; Tragust 2016).

29 In this paper, we document the metapleural gland development in all castes of the
30 dolichoderine ant *Technomyrmex vitiensis* Mann, 1921. *T. vitiensis* is probably of South-
31 East Asian origin, but has recently been found to be widespread in greenhouses across
32 the world (Bolton 2007; Pospischil 2011). The species nests in various materials and
33 spaces such as in leaf litter, under stones, on vegetation, in twigs or under leaves. It tends
34 homopterans for honeydew and preys on small arthropods (Bolton 2007), but its biology in
35 terms of brood care, colony structure and dispersal is rather poorly known and is
36 considered to be similar to its more intensively studied relatives from the *Technomyrmex*
37 *albipes* species group such as *T. brunneus* Forel, 1895, *T. pallipes* (Smith, 1876) and *T.*
38 *difficilis* Forel, 1892. As in several other *Technomyrmex* species, several castes are known
39 in *T. vitiensis*: large, initially winged queens, ergatomorph wingless intercastes, workers,
40 winged males and ergatomorph wingless males (Pech and Bezděk 2016).

41 **Material and Methods**

42 Individuals used for this study were taken from a colony fragment collected in the Prague
43 Zoo (Czech Republic, Praha, 50° 7' N, 14° 24' E) and in the botanical garden of Charles
44 University (Czech Republic, Praha, 50° 4' N, 14° 25' E) in October and November 2015.

45 For histological examination, the posterior thorax half of winged queens and intercastes,
46 workers, and winged and wingless males (5 individuals of each) was fixed in cold 2%

47 glutaraldehyde in a 0.05M Na-cacodylate buffer. After postfixation with 2% osmium
48 tetroxide in the same buffer, tissues were dehydrated in a graded acetone series and
49 embedded in Araldite. Serial semithin sections of 1 μm were made with a Leica EM UC6
50 ultramicrotome, stained with methylene blue and thionin, and viewed in an Olympus BX-51
51 microscope. To estimate the number of secretory cells, we first measured the average cell
52 diameter in each caste. For each caste, we then counted the cumulative number of cells
53 occurring on serial sections through the posterior thorax at an interval equivalent to the cell
54 diameter in that caste. Atrium volume was calculated using the formula of an ellipsoid ($\frac{4}{3}$
55 $\times \pi \times L \times W^2$, with L=length and W=width), as this most closely resembles the atrium
56 shape.

57 For scanning microscopy, the posterior thorax part of 1-3 specimens of each caste were
58 critical point dried in a Balzers CPD 030 instrument, coated with gold and examined in a
59 JEOL JSM-6360 scanning microscope.

60 The statistical differences of cell numbers and diameters as well as atrium measurements
61 among castes were tested using a Kruskal-Wallis test with the following post-hoc
62 comparisons. We performed our calculations using both glands of each individual
63 separately as well as using the means of both glands together, as glands of the same
64 individual are likely not independent. As the results of both analyses were very similar, only
65 the results from the larger data set are presented, providing a better image of the
66 variability of gland morphology.

67 **Results**

68 As in other dolichoderine ants (Yek and Mueller 2011), the paired metapleural gland of
69 *Technomyrmex vitiensis* corresponds with the 'one-chamber design', in which the
70 secretory cells open directly into a sclerotized atrium, that in turn opens to the outside. The

71 gland belongs to class-3 according to the standard classification by Noirot and Quennedey
72 (1974), which means that each secretory cell releases its secretory products through its
73 own accompanying duct cell. The junction between both cells is formed by the 'end
74 apparatus', which functions as a draining device to lead the secretory products from the
75 secretory cell into the duct cell. This general anatomical organization is found in the 5
76 castes, although with considerable size differences (Figs 1,2A). The number of secretory
77 cells, expressed per side for one gland, decreases in the following order: queens ($26.9 \pm$
78 4.9 cells, $n = 8$ glands, Fig. 1A), intercastes (16.8 ± 2.6 cells, $n = 8$ glands, Fig. 1B),
79 workers (10.8 ± 2.6 cells, $n = 6$ glands, Fig. 1C), wingless males (4.3 ± 0.8 cells, $n = 6$
80 glands, Fig. 1D) and winged males (1.9 ± 1.0 cells, $n = 10$ glands, Fig. 1E) ($H=35$; $p<0.01$)
81 (Table 1). Also the size of the spherical secretory cells differs significantly ($H=11.95$;
82 $p=0.0178$) (Table 1) with a diameter that ranges between 25 and 29 μm (Fig. 2B).

83 In all five castes, the duct cells open into the heavily sclerotized atrium through a kind of
84 sieve plate. When transversally sectioned, the number of ducts in this region can be used
85 as a good indication for the corresponding number of secretory cells (Figs 1F-I). The rigid
86 atrium wall has a thickness around 10 μm , which is comparable to the thickness of the
87 thoracic exoskeleton. The size of the atrium, however, is different between castes ($H=33.9$;
88 $p<0.01$) (Table 1, Fig. 2C). Queens have the largest atrium (approx. 80 x 65 μm), followed
89 by intercastes (approx. 75 x 55 μm), workers (approx. 60 x 40 μm), wingless males
90 (approx. 45 x 25 μm) and winged males (approx. 30 x 15 μm). Although the atrium shape
91 is ellipsoid, it varies from more rounded in queens (length/width ratio around 1.2) to more
92 elongated in winged males (length/width ratio around 2) ($H=20.23$; $p<0.01$) (Table 1, Fig.
93 2D).

94 The external opening of the gland is clearly visible in all castes. In the female castes, it
95 appears as an ovoid chamber of roughly 75 x 55 μm in queens and 60 x 40 μm in
96 intercastes and workers, while the male castes have a circular opening with a diameter

97 around 40 μm in wingless males and 30 μm in winged males (Fig. 3). Stiff hairs with a
98 length of 20-30 μm line the outer margin of the gland opening and project towards the
99 opposite side, thus giving the opening a lattice-like appearance, which is more pronounced
100 in the three female than in the two male castes (Fig. 3). Although scanning microscopy
101 best reveals these margin-lining hairs, similar hairs also occur inside the atrium in all
102 castes except the winged males (shown only for queens in Fig. 1A).

103 Discussion

104 Our examination of both internal (histology) and external structure (scanning microscopy)
105 shows that all five castes of *Technomyrmex vitiensis* have a metapleural gland, although
106 with a clear gradient from queens (most developed) via intercastes, workers and wingless
107 males to winged males (least developed). This gradient is reflected in the number and size
108 of the secretory cells, the size of the atrium and the size of the external opening of the
109 gland.

110 The two most commonly listed functions for the metapleural gland are the production of
111 antibiotic substances and chemical defence, although other functions may also be possible
112 (Yek and Mueller 2011). The large opening of the gland to the exterior near the
113 implantation of the hindlegs allows free release of the antibiotic secretion, which is then
114 distributed over the body by leg grooming. The atrium often contains stiff hairs that act as
115 dispenser bristles that guide the secretion to the outside (Hölldobler and Engel-Siegel
116 1984). An elegant study by Fernández-Marín et al. (2006) confirms the general antibiotic
117 nature of the secretion, but shows that ants actively increase grooming and hence
118 distribution of secretion when the degree of infection is higher. Chemical defence is known
119 in some southeast Asian *Crematogaster* species, in which the metapleural gland is
120 hypertrophied with a very large atrium and more than 700 secretory cells per side (Billen et

121 al. 2011). The much smaller gland in *T. vitiensis* makes such defensive function not very
122 plausible, and may rather assume that the gland serves its main role of producing
123 antibiotics. This, however, remains at the hypothetical level as no functional study on the
124 metapleural gland secretion has been done so far.

125 Most research on metapleural gland morphology has been done in the worker caste, in
126 which a correlation has been reported between gland size, as expressed by the number of
127 secretory cells, and body size. This correlation exists both intraspecifically in polymorphic
128 species as leaf-cutting ants (Schoeters and Billen 1993; Bot and Boomsma 1996), as well
129 as between species (Angus et al. 1993). Among all species for which information on
130 metapleural gland cell numbers are available, *T. vitiensis* very much ranks at the low end
131 of the spectrum as its workers with only 10.8 ± 2.6 secretory cells per side are even below
132 *Aneuretus simoni*, that with 14 cells had the lowest number among workers of 44 species
133 covering all major ant subfamilies (Hölldobler and Engel-Siegel 1984). Cell counts for
134 Dolichoderinae in this paper are only available for *Conomyrma bicolor* (125 cells) and
135 *Iridomyrmex purpureus* (317 cells). Fanfani and Valcurone Dazzini (1991) studied the
136 metapleural gland of 6 dolichoderine species, and report they have large glands. The
137 external orifice of the gland in Dolichoderinae is even larger than that of other subfamilies
138 (Maschwitz 1974; Fanfani and Valcurone Dazzini 1991). Why *T. vitiensis* workers have
139 such small metapleural glands remains an open question. It has been reported that the
140 gland is reduced or absent in some arboreal species (Hölldobler and Engel-Siegel 1984),
141 although *T. vitiensis* is not a true arboreal ant (Bolton 2007). It prefers to nest in plant
142 cavities and under leaves, at least in greenhouses (Oettler and Heinze 2009; Pech and
143 Bezděk 2016).

144 Among the various castes in *T. vitiensis*, queens clearly have larger metapleural glands,
145 which is line with the few other species for which gland size in queens and workers has
146 been compared. These data show the queens to have 2-3 times more cells than workers,

147 as reported by Hölldobler and Engel-Siegel (1984) for *Onychomyrmex hedleyi* (102 vs 36),
148 *Pheidole embolopyx* (65 vs 31), *Solenopsis fugax* (178 vs 82) and *Nothomyrmecia*
149 *macrops* (439 vs 272), and by Angus et al. (1993) for *Myrmecia gulosa* (4823 vs 1920) and
150 *M. urens* (381 vs 184). The latter authors speculate that queens have more developed
151 metapleural glands because they have to make the brood chamber as aseptic as possible.
152 The intercastes of *T. vitiensis* are also intermediate between queens and workers in terms
153 of their metapleural gland development. Intercastes are similar in body size to workers, but
154 have considerably more ovarioles (Oettler and Heinze 2009), which may link their more
155 developed metapleural gland rather with their higher egg-laying capacity.

156 Absence or reduced development of the metapleural gland in males was already noticed
157 by Brown (1968) and Hölldobler and Engel-Siegel (1984), and has been interpreted by
158 their short live and hence low contribution to nest sanitation (Stürup et al. 2014). In a
159 number of species with high male/worker ratios and/or males that stay longer in the nest,
160 however, males do have larger metapleural glands, while also a secondary function in sex
161 pheromone production may be possible (Hölldobler and Engel-Siegel 1984). In spite of
162 *Technomyrmex vitiensis* having workers with the lowest number of metapleural gland cells,
163 the gland is also present in the males. This species moreover contains both winged and
164 wingless males (Pech and Bezděk 2016), of which the gland in the wingless individuals
165 has approx. 4 cells per side, while the winged males only have 2 cells on average.
166 Although both male morphs have a very small atrium, the wingless males do have
167 dispenser bristles inside the atrium, whereas these are absent in the winged males.
168 Wingless males live in the colony during the whole year in some *Technomyrmex* species
169 including *T. vitiensis*, often in large numbers (Tsuji and Yamauchi 1994; Yamauchi et al.
170 1991; Pech and Bezděk 2016). These males were not observed to participate in brood
171 care, but their metapleural gland secretion may contribute to the antimicrobial defense of
172 the colony. On the other hand, winged males stay in the colony only for a short time. Also

173 the life expectancy of wingless males is longer than that of winged males (Tsuji and
174 Yamauchi 1994; Pech and Bezděk 2016). Thus, the development of metapleural glands in
175 both male morphs may illustrate their contribution to the colony sanitation, their life
176 expectancy, or both.

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Fig. 1. Semithin cross sections through the metapleural gland: **A**. General view of thorax showing both glands in alate queen (at: atrium, db: dispenser bristles, DC: duct cells, FM: flight muscles, MG: metathoracic ganglion, oe: oesophagus, SC: secretory cells). **B-E**: Representative views of the right metapleural gland in the other castes (A-E all shown at same magnification). **F-I**: Cross section details of duct cells in region where ducts converge before opening into the atrium. Small white arrows indicate end apparatus in secretory cells.

Fig. 2. Graphical comparison of the metapleural gland characteristics in the various castes (Q: queens, IC: intercastes, W: workers, M⁻: wingless males, M⁺: alate males): **A**. Number of secretory cells, **B**. Size of secretory cells, **C**. Atrium volume, **D**. Length/width ratio of atrium.

Fig. 3. Scanning micrographs showing the metapleural gland opening in the various castes, seen from behind after removal of the gaster. **A**. General view of the posterior thorax of a queen showing both metapleural gland openings (arrows). **B-E**. Views of the left metapleural gland opening in the other castes (A-E all shown at same magnification and orientation). HC: hindleg coxa, MC: midleg coxa, PET: petiole, PS: propodeal spiracle.

254

255 **Table legend**

256 Table 1. Significance of the differences in selected metapleural gland parameters among
257 the castes of *Technomyrmex vitiensis*. Significant and nearly significant differences are
258 shown in bold.

259 Table 1. Significance of the differences in selected metapleural gland parameters among the castes of
260 *Technomyrmex vitiensis*. Significant and nearly significant differences are shown in bold.

Parameter	Caste	Queen	Intercaste	Worker	Wingless male	Winged male
Cell number	Queen		>>0.05	>>0.05	<0.01	<0.01
	Intercaste	>>0.05		>>0.05	>>0.05	<0.01
	Worker	>>0.05	>>0.05		>>0.05	>>0.05
	Wingless male	<0.01	>>0.05	>>0.05		>>0.05
	Winged male	<0.01	<0.01	>>0.05	>>0.05	
Cell diameter	Queen		>>0.05	>>0.05	0.043	0.063
	Intercaste	>>0.05		>>0.05	>>0.05	>>0.05
	Worker	>>0.05	>>0.05		>>0.05	>>0.05
	Wingless male	0.043	>>0.05	>>0.05		>>0.05
	Winged male	0.063	>>0.05	>>0.05	>>0.05	
Atrium volume	Queen		>>0.05	0.045	<0.01	<0.01
	Intercaste	>>0.05		>>0.05	0.075	<0.01
	Worker	0.045	>>0.05		>>0.05	>>0.05
	Wingless male	<0.01	0.075	>>0.05		>>0.05
	Winged male	<0.01	<0.01	>>0.05	>>0.05	
Width/length ratio atrium	Queen		>>0.05	>>0.05	0.085	<0.01
	Intercaste	>>0.05		>>0.05	>>0.05	<0.01
	Worker	>>0.05	>>0.05		>>0.05	>>0.05
	Wingless male	0.085	>>0.05	>>0.05		>>0.05
	Winged male	<0.01	<0.01	>>0.05	>>0.05	

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