1 **Morphology of the novel ventral scape gland in the ant genus *Strumigenys***

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7 **Keywords:** scape; antenna; histology; ultrastructure; exocrine glands; Formicidae

8 **Abstract**

9 We describe a novel ventral scape gland in all 19 species of the genus *Strumigenys*
10 that we examined, confirming the postulated existence of this gland by Bolton (1999). The
11 gland occurs in workers and queens, it belongs to ‘class-1’ and is formed by a layer of
12 epithelial secretory cells that line the ventrodistal tegumental cuticle of the antennal scape.
13 The gland is characterized by its bowl-shape, by the ultrastructural characteristics of
14 microvillar arrangement and by the presence of nerves in between the epithelial cells. These
15 features make this gland structurally very similar to 3 other epithelial glands that occur
16 exclusively in *Strumigenys*. Besides the epithelial ventral scape gland, also scattered class-
17 3 glandular cells are found. The function of both glands in the scape remains unclear,
18 although a role in prey attraction cannot be excluded.

19

20 Introduction

21 Social insects, and ants in particular, have an extremely developed exocrine system
22 (Billen and Šobotník, 2015). Glands are located all over the body, including the legs,
23 mouthparts and antennae. The first mention of an antennal gland was made by Janet (1894,
24 1898), although the gland cells he described are located inside the head capsule at the base
25 of the antennae in *Myrmica rubra*. This gland was more recently also described in *Eciton*
26 *rapax* queens (Hölldobler, 2016). Intra-antennal glands in ants were first reported in the
27 distal antennomers of queens and workers in *Solenopsis invicta* (Isidoro et al., 2000). These
28 glands are formed by class-3 secretory cells following the classification by Noirot and
29 Quennedey (1974). Their accompanying ducts open through small pores that are arranged
30 as a ring along the smooth proximal portion of the terminal antennomers (Isidoro et al., 2000).
31 A comparative survey for this gland in 41 ant species belonging to 13 genera confirmed its
32 existence only in all 26 examined *Solenopsis* species and also in *Tetramorium bicarinatum*
33 (Renthal et al., 2008). In *Dinoponera lucida*, Marques-Silva et al. (2006) found class-3 as
34 well as epithelial class-1 glands (Noirot and Quennedey, 1974) in all antennomers, but not
35 in the scape. The presence of antennal glands in the scape of ants was first documented in
36 *Eciton burchelli* workers with the description of a novel scape gland which consists of a
37 cluster of class-3 gland cells at the dorsal distal tip of the scape (Billen, 2000). Later, Romani
38 et al. (2006) observed a similar gland also in the slightly swollen distal portion of the scape
39 in the female castes of the slave-making *Polyergus rufescens* as well as its slave species
40 *Formica cunicularia*.

41 Based on SEM solely and exclusively restricted to species of the genus *Strumigenys*,
42 Bolton (1999) described “a circular, oval or more elongated patch of pale tissue” at the distal
43 ventral side of the scape. Without any histological checking, he concluded that this region
44 corresponds with a novel exocrine structure and named it ventral scape gland. Silva and
45 Feitosa (2019) mention that in some *Strumigenys* species this patch is unsculptured while
46 in others it can appear translucent.

47 *Strumigenys* is a genus of mainly subtropical and tropical myrmicine ants that live in
48 small colonies in the leaf-litter. They are slow-moving predators with either short or long
49 mandibles that mainly feed on collembola and other small arthropods (Masuko, 1984;
50 Dejean, 1985). Through histologically surveying the scape of several *Strumigenys* species,
51 we aimed to verify the existence of the presumed ventral scape gland, and here report on
52 our affirmative findings.

53 **Materials and methods**

54 *Strumigenys* ants for this study were obtained from field colonies of 19 species from
55 various localities mostly in Taiwan (Table 1). To allow the various chemicals used during
56 tissue processing to easily enter the antennal scape, we separated the anterior head from
57 the body by making a transverse cut at the level of the compound eyes, and also cut off the
58 distal antennomeres. The scape of these small ants was left attached to the head during the
59 various steps of tissue processing to avoid it would otherwise be lost, and was only detached
60 from the head at the moment of embedding. Tissues were fixed with 2.5% cold
61 glutaraldehyde buffered in 0.1 M Na-cacodylate (pH 7.2) for 12 hours. Postfixation was
62 carried out with 2% cold osmium tetroxide for 1 hour, and was followed by dehydration in a
63 graded acetone series and embedding in Araldite. Blocks were sectioned with a Leica EM
64 UC6 ultramicrotome at a thickness of 1 μm and stained with 1% methylene blue and thionin.
65 Serial semithin sections were examined with an Olympus BX-51 microscope connected to
66 a camera. Double-stained 70 nm thin sections were studied under a Zeiss EM900 electron
67 microscope. Samples for scanning microscopy were coated with palladium and viewed
68 under a JEOL JSM-6360 scanning microscope. Behavioural observations and video
69 imaging were made through a Leica S8APO stereomicroscope connected to a Panasonic
70 Lumix DMC-GH1 camera.

71 **Results**

72 Scanning microscopy of workers and queens confirmed the existence of an elongate
73 ventrolateral patch at the distal part of the scape, as mentioned by Bolton (1999). This
74 appears as an apparently smooth area without any sculpturation or hairs as is the case
75 elsewhere on the antennal surface (Fig. 1A). Close-up examination, however, revealed that
76 the patch is characterized by numerous minute depressions on the cuticular surface. These
77 appear as very small slightly elongate slits with a size around 100 nm and a density of 1
78 slit/ μm^2 (Fig. 1B,C).

79 Histological semithin sections through the distal part of the scape in both workers and
80 queens showed the presence of a conspicuous glandular epithelium underneath the
81 ventrolateral cuticle (Fig. 2A-F). The epithelium with basally located round nuclei has a
82 thickness of 7-10 μm , whereas epidermal thickness underneath the cuticle elsewhere in the
83 scape hardly measures between 1 and 3 μm . In contrast, the cuticle overlaying the glandular
84 epithelium has approx. half the thickness (3-4 μm) than that of the scape cuticle elsewhere
85 (8-10 μm), resulting in the scape gland having a bowl-shaped appearance on sections. Apart
86 from the epithelial ventrodistal scape gland of class-1, also scattered class-3 secretory cells

87 with a diameter of approx. 10 μm can be found (Fig. 2E). Serial sections through the male
88 scape samples that we had available (of *S. benten*) did not show any trace of glandular
89 tissue (Fig. 2G).

90 Electron microscopy revealed that the cuticle overlaying the glandular epithelium has
91 an irregular texture with transversely oriented whorl-like connections that end up in very
92 small pores of approx. 100 nm at the surface (Fig. 3A,B). The cytoplasm contains numerous
93 round to elongate vacuolar spaces in its apical region (Fig. 3A,B). The spaces are
94 associated with microvilli, although these do not reach up to the overlaying cuticle, and thus
95 leave thin cytoplasmic processes underneath the cuticle (Fig. 3C,D). Other cytoplasmic
96 elements include Golgi apparatus (Fig. 3E) and numerous mitochondria (Fig. 3F). We did
97 not find endoplasmic reticulum, neither of the smooth nor of the granular type. Wedged in
98 between the epithelial gland cells we observed a small nerve fibre (0.5-1 μm) that is loaded
99 with dark neurosecretory vesicles of 50-80 nm diameter (Fig. 3F). Besides the epithelial
100 ventral scape gland, the scape also contains scattered class-3 gland cells (Fig. 4A-D), that
101 are connected with their accompanying duct cells that open at the scape surface as isolated
102 pores. The secretory cells have a diameter between 5 and 10 μm and are clearly
103 recognizable by their end apparatus and the presence of a Golgi apparatus (Fig. 4A-D).

104 Behavioural observations (of *Strumigenys lacunosa* and *S. orchidensis*) showed that
105 workers in resting position or during normal walking hold their mandibles almost closed and
106 their antennae under an angle of less than 180 degrees (see Video 1 in Supplementary
107 material). During prey hunting and defence, however, both the mandibles and antennae are
108 held at an angle of 180 degrees or more, which involves an upward tilting of the scape that
109 exposes the area of the ventral scape gland towards the prey or opponent (see Video 2 in
110 Supplementary material).

111 Discussion

112 We confirm the presence of an epithelial ventral scape gland in all 19 examined
113 *Strumigenys* species, for the first time bringing empirical evidence of this gland's existence
114 as postulated by Bolton (1999). This confirmation therefore adds a novel structure to the
115 already impressive exocrine system of ants. It is amazing that Bolton's conclusion is based
116 on scanning microscopy observation merely, and equally amazing that his reporting was
117 only formulated in text without any reference to illustrations. His description of the smooth
118 patch on the ventrodistal scape cuticle was probably based on examination at a rather low
119 magnification, as he did not mention the presence of numerous minute slits around 100 nm
120 in the smooth area. Such extremely small depressions in the cuticular surface have also

121 been observed in the apicofemoral and apicotibial (Billen et al., 2000) and basimandibular
122 gland of *Strumigenys* (Wang et al., 2021), several other epithelial leg glands of ants (Billen,
123 2009) and in the mandibular pit gland of *Brachyponera sennaarensis* (Billen and Al-Khalifa,
124 2016). These abundant minute slits represent cuticular cracks through which the secretions
125 of class-1 glands can reach the surface, and are totally different from the larger round pores
126 with a characteristic diameter of 0.5-1 μm that are the duct openings of class-3 glands.

127 The reduction in cuticle thickness of the part that overlays the glandular epithelium to
128 half the size it has elsewhere in the scape gives the gland a bowl-shaped appearance. Two
129 additional ultrastructural peculiarities make the ventral scape gland look very similar to the
130 basimandibular gland (Wang et al., 2021) and the apicofemoral and apicotibial glands (Billen
131 et al., 2000; Billen, 2009) that represent other epidermal glands with exclusive occurrence
132 in *Strumigenys*: microvilli make no direct contact with the overlaying cuticle as is usual for
133 class-1 glands (Noirot and Quennedey, 1974; Billen and Morgan, 1998), but are confined to
134 large vacuolar spaces in the apical part of the cells. As a result, thin subcuticular cytoplasmic
135 processes occur, of which the functional significance as yet remains unclear. The other
136 feature the ventral scape gland has in common with the epidermal glands in *Strumigenys* is
137 the occurrence of a nerve fibre in between the glandular cells. This indicates the existence
138 of a neurosecretory control, although the precise mechanism of secretion release remains
139 unknown, as there are no muscles directly associated with these glands.

140 Although glands are frequently found in the flagellar part of insect antennae, their
141 presence in the scape is far less common (Romani et al., 2003, 2006; Marques-Silva et al.,
142 2006). In the few examples where scape glands were found, they all belong to class-3 and
143 occur in males of parasitoid wasps, with a function in courtship behavior (Dahms, 1984;
144 Shirley et al., 2019). Our preliminary notice of the ventral scape gland being absent in males
145 and especially its common presence in workers make such reproduction-related function in
146 *Strumigenys* obviously not relevant. The presence of gland cells, albeit of class-3, in the
147 ventrodistal tip of the scape in workers and queens of *Polyergus rufescens* may be linked
148 with its life as a slave-making social parasite, in which the young queen has to penetrate a
149 host colony during colony foundation and the workers have to enter other colonies of the
150 host species later on to steal cocoons (Romani et al., 2006). That also workers of the host
151 species *Formica cunicularia* have similar scape glands can be understood in the context of
152 manipulation of host behaviour (Romani et al., 2006). Among our study species,
153 *Strumigenys mutica* is also a social parasite (Wang et al., 2021). As this is not a slave-
154 making species, however, it is only the queen that invades a host colony. We moreover

155 found the ventral scape gland in the parasite (*S. mutica*), the host species (*S. formosensis*
156 and *S. solifontis*), as well as in 16 other *Strumigenys* species, which does not make a
157 connection with social parasitism very likely. It is noteworthy, however, that the novel
158 basimandibular gland is clearly more developed in *S. mutica* queens than in workers (Wang
159 et al., 2021).

160 It remains as yet unclear what function the ventral scape gland has. *Strumigenys* ants
161 feed on collembola and other small arthropods, which represents an apparent contradiction
162 as the ants are slow-moving whereas their prey is usually fast. Masuko (1984) and Dejean
163 (1985) speculated that labral secretions and/or the spongiform tissue on the petiole and
164 postpetiole could play a role in prey attraction. Bolton (1999) even attributes a possible
165 function in such allomone production to the presumed ventral gland of the scape. The higher
166 angle at which the mandibles and antennae are held during prey hunting and defensive
167 behaviour, as observed when filming the ants, goes along with an upward tilting of the
168 scapes. This results in a more forward exposure of the region of the ventral scape gland in
169 the direction of the prey or opponent, which is suggestive for an eventual function in prey
170 attraction. In this case, secretion may reach the scape's ventrodistal surface through the
171 minute slits, and from there may evaporate.

172 Whereas our finding of the distoventral epithelial gland represents the first report of a
173 class-1 gland in the scape, the presence of class-3 gland cells has been noticed previously
174 in males of some parasitoid wasps (Dahms, 1984; Shirley et al., 2019) and in workers and
175 sometimes also queens of a few ant species (Billen, 2000; Romani et al., 2006). These
176 observations describe the clustered occurrence of up to 20 gland cells in the distal portion
177 of the scape, while we only found few isolated cells that are scattered in the scape of
178 *Strumigenys*. The cells we found are therefore probably not homologous to these
179 anatomically well-defined and clustered gland cells in other ants, but may form part of the
180 scattered subepithelial glands reported by Gobin et al. (2003), and that may modify the
181 composition of the cuticular surface. We have no indication as yet on the function these
182 scattered gland cells on the scape of *Strumigenys* ants may have.

183 **Acknowledgements**

184 We are very grateful to An Vandoren for assisting in section preparation and to Alex
185 Vrijdaghs for his help in scanning microscopy. We thank Fuminori Ito for providing the
186 specimens of *Strumigenys hexamera* and two anonymous reviewers for their constructive
187 comments on the manuscript. CW acknowledges a PhD scholarship grant (No.
188 201906300043) by the China Scholarship Council (CSC).

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250 Table 1. Survey of ants studied with their collection locality and indication of number of
 251 workers (W), alate queens (AQ) and males (M) examined.

	species	collection locality	W	Q	M
short-mandibulate	<i>S. benten</i>	Yuchi Township, Nantou County, Taiwan	2	0	2
	<i>S. canina</i>	Kobe, Japan	1	0	0
	<i>S. elegantula</i>	Yuchi Township, Nantou County, Taiwan	0	1	0
	<i>S. emmae</i>	Baihe District, Tainan City, Taiwan	2	1	0
	<i>S. hexamera</i>	Kagawa, Japan	5	0	0
	<i>S. kichijo</i>	Taipei, Taiwan	4	0	0
	<i>S. leptothrix</i>	Ren'ai Township, Nantou County, Taiwan	5	0	0
	<i>S. mutica</i>	Yuchi Township, Nantou County, Taiwan	7	3	0
	<i>S. sauteri</i>	Lugu Township, Nantou County, Taiwan	2	2	0
long-mandibulate	<i>S. chuchihensis</i>	Jianshi Township, Xinchu County, Taiwan	2	0	0
	<i>S. formosensis</i>	Ren'ai Township, Nantou County, Taiwan	7	0	0
	<i>S. hispida</i>	Yuchi Township, Nantou County, Taiwan	3	0	0
	<i>S. lacunosa</i>	Lugu Township, Nantou County, Taiwan	6	1	0
	<i>S. liukueiensis</i>	Jiji Township, Nantou County, Taiwan	1	3	0
	<i>S. minutula</i>	Hengchun Township, Pingtung County, Taiwan	1	0	0
	<i>S. nanzanensis</i>	Lanyu Township, Taitung County, Taiwan	2	0	0
	<i>S. orchidensis</i>	Lanyu Township, Taitung County, Taiwan	1	0	0
	<i>S. rogeri</i>	Jiji Township, Nantou County, Taiwan	1	1	0
	<i>S. solifontis</i>	Lugu Township, Nantou County, Taiwan	5	1	0

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256 Fig. 1. Scanning micrographs illustrating the position of the ventral scape gland. **A.** Ventral
257 view junction between scape (S) and antennal pedicel (P) of *S. formosensis* worker,
258 asterisk indicates smooth area where gland occurs. **B.** Detail view of smooth area of *S.*
259 *leptothrix* worker, framed area appears enlarged in **C.** Note occurrence of numerous
260 minute depressions on cuticular surface.

Fig. 2. Semithin sections through head and scape (A-D: transverse, E-G: longitudinal, distal part to the left). **A**. Posterior head region of *S. formosensis* worker with scape (S) and antennal flagellum (F). Black arrows indicate position of ventral scape gland. Close-up views show epithelium of ventral scape gland (white arrowheads) in *S. solifontis* worker (**B**), *S. liukueiensis* alate queen (**C**), *S. mutica* alate queen (**D**), *S. mutica* worker (**E**) and *S. sauteri* worker (**F**). Note class-3 gland cell with duct in proximal scape region of *S. mutica* worker (black arrowhead). **G**. Short scape of *S. benten* male, no ventral scape gland present. an: antennal nerve, Br: brain.

Fig. 3. Electron micrographs of ventral scape gland. **A-B**. Glandular epithelium in worker of *S. lacunosa* (**A**) and *S. chuchihensis* (**B**), showing microvilli (mv), vacuolar spaces (vs) and basally located nucleus (N). Note small depressions at cuticular surface (black arrowheads). AN indicates antennal nerve, NF is small nerve fibre wedged in between gland cells. **C-D**. Detail of microvilli and subcuticular processes (white arrowheads) in *S. lacunosa* worker (**C**) and *S. solifontis* alate queen (**D**). **E-F**. Central cytoplasm in worker of *S. mutica* (**E**) and *S. lacunosa* (**F**), showing mitochondria (M), Golgi apparatus (GA) and nerve fibre wedged between gland cells (NF).

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Fig. 4. Electron micrographs of class-3 scape glands in: **A.** *S. mutica* alate queen, **B.** *S. mutica* worker, **C.** *S. hispida* worker, **D.** *S. lacunosa* worker. ct: cuticle, DC: duct cell, EA: end apparatus, GA: Golgi apparatus, N: nucleus.

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Supplementary material

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Video 1: *S. lacunosa* worker during normal walking (real speed video). Note almost closed mandibles and antennae opened at an angle less than 180 degrees.

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Video 2: *S. lacunosa* worker during prey hunting (real speed video). Note both antennae and mandibles opened at an angle of 180 degrees or more, and upward tilting of the antennae.