Myrmecological News | 19 | 61-64 | Vienna, January 2014

A novel intramandibular gland in the ant *Tatuidris tatusia* (Hymenoptera: Formicidae)

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Abstract

The mandibles of *Tatuidris tatusia* workers are completely filled with glandular cells that represent a novel kind of intramandibular gland that has not been found in ants so far. Whereas the known intramandibular glands in ants are either epithelial glands of class-1, or scattered class-3 cells that open through equally scattered pores on the mandibular surface, the ducts of the numerous class-3 secretory cells of *Tatuidris* all converge to open through a conspicuous sieve plate at the proximal ventral side near the inner margin of each mandible.

Key words: Exocrine glands, mandibles, histology, Agroecomyrmecinae.

Myrmecol. News 19: 61-64 (online 16 August 2013)
ISSN 1994-4136 (print), ISSN 1997-3500 (online)

Received 31 May 2013; revision received 5 July 2013; accepted 16 July 2013
Subject Editor: Alexander S. Mikheyev

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Introduction

Ants are well known as walking glandular factories, with an impressive overall variety of 75 glands recorded so far for the family (Billen 2009a). The glands are not only found in the head, thorax and abdomen, but also occur in the appendages such as the legs, that can contain up to 20 different glands (Billen 2009b), the antennae (Billen 2000, Isidoro & al. 2000), and the mandibles. Intramandibular glands were first described by Schoeters & Billen (1994) as a quite common exocrine structure in ants that was found in almost all examined species representing ten subfamilies. They correspond with class-3 according to the classification of Noirot & Quennedey (1974), and are thus formed by various bicellular units, each unit comprising a rounded secretory cell and an accompanying duct cell. The duct cells open through single scattered pores on the mandibular surface (Schoeters & Billen 1994, Grasso & al. 2004, Roux & al. 2010). Besides this common intramandibular gland that is composed of class-3 cells, epithelial intramandibular glands composed of cylindrical class-1 cells may also be found (Billen & Espadaler 2002 in *Strumigenys membranifera*, Amaral & Caetano 2006 in *Atta sexdens*, Martins & Serrão 2011 in *Hypoponera* sp. and *Pachycondyla* spp., Billen & al. 2013 in *Protanilla wallacei*).

The "armadillo ant" *Tatuidris* is a Neotropical genus that was first described by Brown & Kempf (1968) as a new taxon in the Myrmicinae. It was later raised to become the only extant genus of the new subfamily Agroecomyrmecinae (Bolton 2003, Donoso 2012). The genus is currently considered monotypic (Donoso 2012). Very recent behavioural observations and stable isotope analysis revealed that *T. tatusia* is a top predator of the leaf-litter food web (Jacquemin & al. in press). We took advantage of the availability of two live specimens to carry out a first study of the internal morphology in the Agroecomyrmecinae. Our survey confirms the presence in *T. tatusia* workers of the standard exocrine glands that are known in ants (mandibular, pro- and postpharyngeal glands in the head, labial and metapleural glands in the thorax, venom and Dufour glands in the abdomen), but also revealed the occurrence of a novel kind of intramandibular gland, which we here describe.

Material and methods

Tatuidris tatusia workers were collected in the Copalinga Private Reserve, Ecuador (4° 5' S, 78° 57' W) at 1050 m above sea level. Previous sampling indicated that *T. tatusia* was relatively common at this locality (Jacquemin & al. in press). To capture this elusive ant, all the leaf litter present inside five 1-m^2 quadrats was transferred to Winkler extractors for 24h and ants were collected live in plastic bags containing humid paper (Silva & Brandão 2010). Our collection provided us with two workers that were used for histological analysis, while one worker previously sampled from the same locality and preserved in ethanol (Jacquemin & al. in press) was used for scanning electron microscopy. For histology, the anterior portion of the heads of the two live workers were fixed in 2% glutaraldehyde and postfixed in 2% osmium tetroxide. Dehydration took place in a graded acetone series. The Araldite-embedded blocks were sectioned with a Leica EM UC6 ultramicrotome. Semithin 1 µm sections were stained with methylene

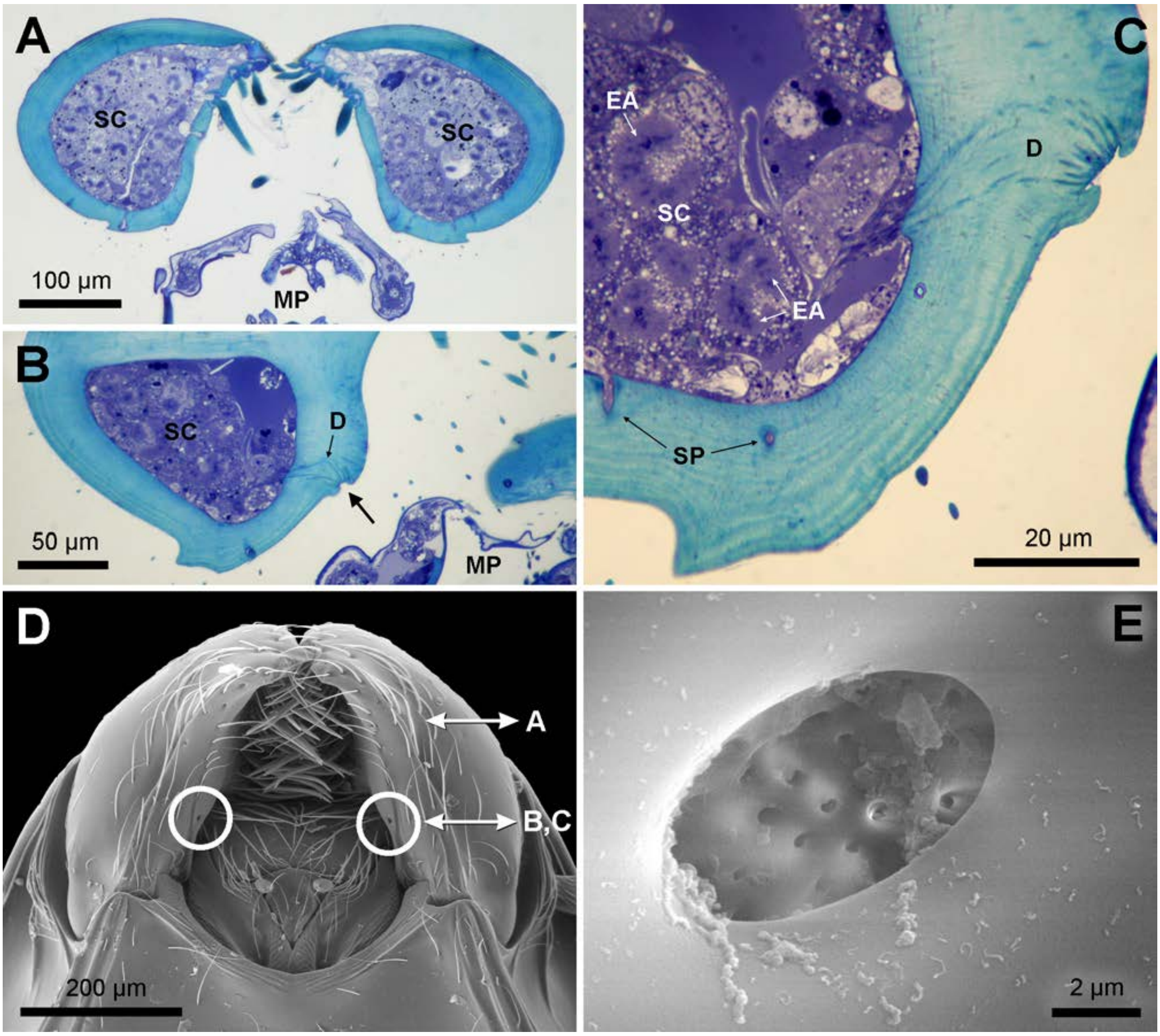

Fig. 1: (A) Cross section through the distal part of the mandibles, showing their almost complete filling with secretory cells. (B) Cross section view in proximal part of left mandible, showing the area of the sieveplate (arrow). (C) Detail of sieveplate area. (D) Scanning micrograph of the anterior head part in ventral view. Encircled areas indicate sieveplate on both mandibles; double arrows indicate sectioning level of figures A, B and C. (E) Scanning micrograph detail of sieveplate, showing opening of duct cells. D: ducts, EA: end apparatus, MP: mouthparts, SC: secretory cells, SP: sensillar protrusions.

blue and thionin and examined in an Olympus BX-51 microscope. Heads for scanning microscopy were detached from the body, gold-coated in a Bal-Tec Sputter Coater SCD 050 and examined in a FEI Quanta 200 scanning electron microscope.

Results

Tatuidris tatusia workers are characterized by a rounded head with non-overlapping triangular mandibles. Serial sections through the anterior head portion of both *T. tatusia* workers revealed that the mandibles are almost completely filled with rounded secretory cells with a diameter of approx. 25 - 30 µm (Figs. 1A - C). The ovoid nucleus measures approx. 10 × 5 µm (length × width), and occurs eccentrically in the cell. A major part of the cell is occupied by the dark-staining and often curved end apparatus (Fig. 1C). As we did not have material available for electron microscopy, further cytoplasmic details unfortunately cannot be given. From each secretory cell, the end apparatus continues in a slender duct with a diameter around 0.5 µm. The ducts do not open as scattered pores on the mandibular surface, as is known for the intramandibular gland of other ants (SCHOETERS & BILLEN 1994), but converge to form a conspicuous bundle that opens through a sieveplate at the proximal ventral side near the inner margin of each mandible (Figs. 1B - C). The sieveplate occurs at the bottom of a cuticular depression of which the opening is lined by an over-hanging rim (Fig. 1C). Scanning microscopy shows the openings at the proximal ventral side of both mandibles (Fig. 1D), with a clear appearance of the sieveplate (Fig. 1E). Approx. 20 - 25 duct openings with a diameter around 0.5 µm can be seen, although each sieveplate will contain a higher number of pores, as the circular rim obstructs viewing the laterally occurring pores underneath.

Discussion

The intramandibular gland we here describe in workers of *Tatuidris tatusia* belongs to class-3 according to the classification of NOIROT & QUENNEDEY (1974), with rounded secretory cells that are accompanied by a slender duct cell. In contrast to the commonly known intramandibular class-3 gland in ants, with scattered secretory cells and single duct openings on the mandibular surface (SCHOETERS & BILLEN 1994), the gland in *T. tatusia* differs by the mandibles being almost completely filled with secretory cells, and especially by the grouped occurrence of the ducts, that open through a conspicuous sieveplate. These clearly different characteristics lead us to consider the intramandibular gland of *T. tatusia* as a separate exocrine structure, and thus as a putative autapomorphy for the genus. The phylogenetic position of *Tatuidris* within Formicidae is still unclear. Morphological studies suggest this genus may belong to the Myrmicinae (BARONI URBANI & DE ANDRADE 2007) or be its sister lineage (KELLER 2011). Molecular studies, however, place it as sister lineage to Amblyoponinae (RABELING & al. 2008) or to Paraponerinae (BRADY & al. 2006, MOREAU & al. 2006, MOREAU & BELL in press). In the latter case, the clade Paraponerinae+Agroecomyrmicinae is sister to Ponerinae. It therefore would be interesting to look for the sieveplate in other ant taxa as an attempt to detect a synapomorphy shared with *Tatuidris* and, hence, to place this genus with confidence in the phylogenetic tree of Formicidae. For instance, the sieveplate should be looked for in the myrmicine genus *Phalacromyrmex* as DONOSO (2012) suggested that this genus could share morphological characters with *Tatuidris*.

As for the common intramandibular gland with isolated duct openings, it may be difficult to attribute a function to this novel gland in *Tatuidris tatusia*, as both glands lack a reservoir, which compromises collection of reasonable amounts of secretion for testing. Although the diet of *T. tatusia* is totally unknown, this ant is suspected to be a specialist predator on slippery or hairy invertebrates (BROWN & KEMPF 1968, LACAU & al. 2012). A recent stable isotope analysis indicated that *T. tatusia* is one of the top predators of the leaf-litter food web (JACQUEMIN & al. in press). The fact that workers move very slowly suggests that the species is a sit-and-wait predator (JACQUEMIN & al. in press). It is therefore tempting to hypothesize that the intramandibular gland may secrete allomones to attract preys. AMARAL & CAETANO (2006) postulate that the intramandibular gland serves a function related to feeding, as the gland opens at the inner face of the mandible, where it comes in contact with the food ingested. This may also be the case for the novel intramandibular gland of *T. tatusia*, that equally opens in a position where food ingestion occurs. However, no clear function could be found so far for the common intramandibular gland in Formicidae (SCHOETERS & BILLEN 1994, GRASSO & al. 2004, AMARAL & CAETANO 2006, MARTINS & SERRÃO 2011), except in *Oecophylla* weaver ants, where workers display a peculiar mandibular rubbing behaviour for marking and nestmate recruitment (ROUX & al. 2010). Our finding of this novel gland in *T. tatusia* will form a challenge for behavioural research when live material of these elusive ants will again become available.

Acknowledgements

We very much acknowledge the help of An Vandoren, Julien Cillis and Diego Marin in section preparation, scanning microscopy, and fieldwork, respectively. We also warmly thank Catherine Vits and Boudewijn De Roover for allowing us to collect ants in their private reserve. The manuscript was improved by comments provided by Alexander Mikheyev, José Eduardo Serrão and one anonymous reviewer. This work received financial support of the Belgian Federal Science Policy Office (BELSPO) and of the Belgian Focal Point to the Global Taxonomy Initiative (GTI).

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