



## Intramandibular glands in ants

Johan BILLEN & Chu WANG

### Abstract

This review documents the occurrence of 11 exocrine glands that have been discovered inside the mandibles of ants in the past three decades. Among these, the ventrolateral intramandibular gland and the distal blade mandibular gland represent novel findings. The high number of intramandibular glands urged for an updated revision of the terminology of gland names that we provide here. Contrary to this diversity, the majority of the glands occurs in a single species or genus and thus reflects a narrow phylogenetic distribution. Glands formed by both epithelial class-1 cells and by bicellular units of class-3 cells are found, but none with reservoirs. Although reports of reservoir-containing epithelial glands in ant mandibles have been published in literature, these represent erroneous observations of sensillar structures. The function of most intramandibular glands remains unknown and requires further study. This review updates the currently known exocrine repertoire of ants to 115 described glands.

**Key words:** Hymenoptera, Formicidae, mandibles, morphology, ultrastructure, review.

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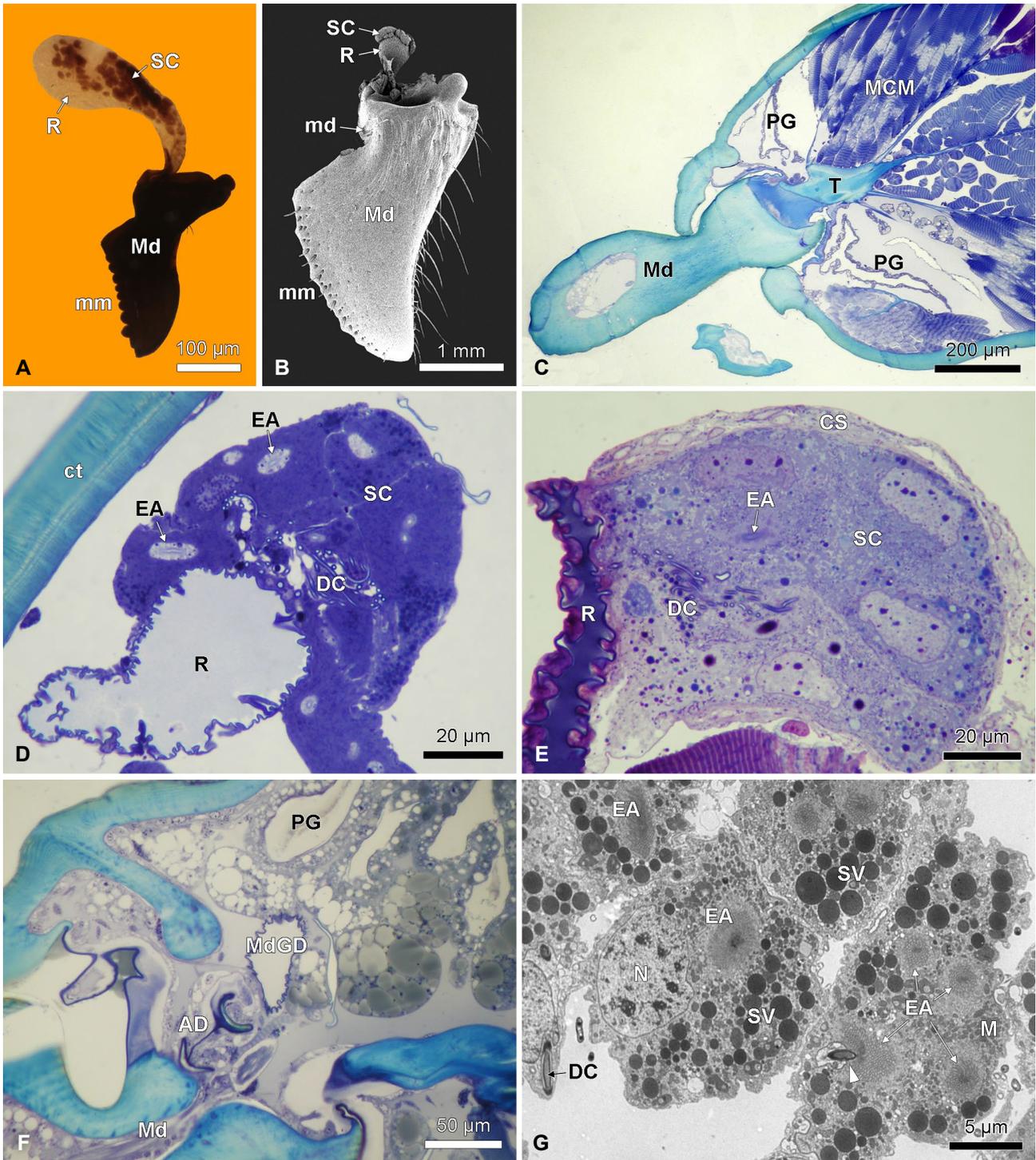
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### Introduction

With 14,312 described species (BOLTON 2024), ants show an extraordinary variety of head shapes and an equally astonishing variety of the anatomy of their mandibles that reflects the large range of functions they perform. Mandibles are used as important tools for food collection and manipulation, ranging from leaf-cutting instruments in the Attini *sensu strictu* to seed-grinding devices in *Pogonomyrmex* harvester ants (TSCHINKEL & KWAPICH 2016). Predatory species show peculiar mandibular modifications to strip prey from entangling bristles (*Thaumatomyrmex*: BRANDÃO & al. 1991) or for handling delicate food items such as the arthropod eggs on which they feed (Proceratiini: MASUKO 2019, 2020). The ultrafast snapping mandibles of trap-jaw species are known to represent the fastest muscular acceleration of resettable animal movements that result from the energy output of closer muscle contraction in combination with the development of power amplification mechanisms (LARABEE & SUAREZ 2014, BOOHER & al. 2021, PATEK 2023). Mandibles can be modified into sickle-shaped fighting tools as in *Eciton* army ant soldiers (O'DONNELL & al. 2018) and *Polyergus* slave-making ants (KWAIT & TOPOFF 1984), or perform peaceful tasks as brood handling and nest construction (arrangement of pine needles in *Formica*: GORYUNOV 2015; wall papering in *Harpegnathos*:

PEETERS & al. 1994; chewing wood in *Melissotarsus*: KHALIFE & al. 2018).

Mandibles considerably vary in size and shape but generally appear as heavily sclerotized structures with proximolateral articulations. In most ants, the mandible consists of a robust proximal stem and a triangular distal blade. The inner side of the blade bears a masticatory margin (Fig. 1A, B) that often bears a series of teeth. Because of their prominent mechanical actions, the tip and biting edge of ant mandibles are often reinforced with zinc to increase abrasion resistance (SCHOFIELD & al. 2021; see gold-stained mandibular tip in several of the histological figures in this review). Although ant mandibles are dicondylic, the dorsal articulation is modified such that the range of motion is not simply transverse or restricted to a plane. The mandibles are moved by the antagonistic effects of the mandibular opener and closer muscles in the head capsule (e.g., PAUL 2001); internally, the mandibles only bear chordotonal muscle fibers (e.g., EHMER & GRONENBERG 1997, GRONENBERG & al. 1998). The opener and closer muscles are attached to the base of the mandible via tendons (LARABEE & al. 2017) as shown in Figure 1C. The precise movements of the mandible, moreover, depend on the form and the mechanism of their articulation to the head capsule (KANG & al. 2023, RICHTER & al. 2023).



**Fig. 1:** Mandibular gland. (A) Dissection view of mandible (Md) with masticatory margin (mm) and attached mandibular gland of *Atta sexdens* worker (R: reservoir, SC: secretory cells). (B) Mandible and attached mandibular gland of *Paraponera clavata* worker (md: mandalus). (C) Longitudinal section through anterior head of *Myopopone castanea* worker, note branched cuticular tendon (T) onto which mandible closer muscles (MCM) attach. PG: pharyngeal gland. (D) Secretory cells, duct cells (DC), and reservoir of mandibular gland of *Myopias emeryi* worker. EA: end apparatus, ct: cuticle. (E) Mandibular gland of *Brachyponera sennaarensis* queen surrounded by connective tissue sheath (CS). (F) Anchor-shaped duct (AD) connecting mandibular gland duct (MdGD) to mandalus (*Cerapachys sulcinodis* worker). (G) Secretory cells of *Camponotus japonicus* worker showing end apparatus, secretory vesicles (SV), and numerous mitochondria (M). Note junction of end apparatus and duct cell (white arrowhead). N: nucleus.

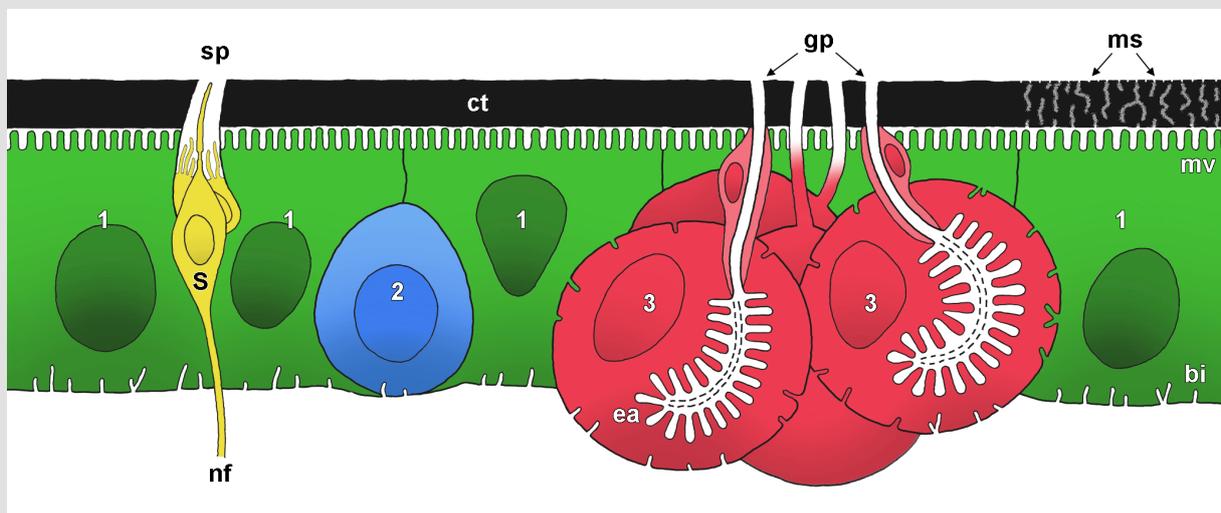
### Box 1: Classification of insect exocrine gland cells

The universally used classification of exocrine gland cells in arthropods was introduced by NOIROT & QUENNEDEY (1974). This system distinguishes three categories according to the cellular organization of the secretory cells, of which class-1 and class-3 cells are by far the most common (Box 1 Fig.). Cells of both categories can occur underneath the body wall as part of the integument and in this case release their secretion directly to the exterior. Alternatively, they can be arranged around a reservoir space in which secretion can be temporarily stored. The latter arrangement, however, does not apply to any of the intramandibular glands because of space constraints.

**Class-1 cells** are directly derived from the integumental epidermis and are arranged as a monolayered epithelium. The cells are apically lined with a layer of cuticle and basally rest on a thin basement membrane (Box 1 Fig., green). The apical cell membrane is usually differentiated into a microvillar brush border to increase the transportation surface through which the glandular secretion leaves the cell; irregular basal invaginations with a similar surface-increasing function may also occur to facilitate the uptake of precursor molecules from the underlying haemolymph. The cuticle forms a mesh through which secretory material can diffuse towards the exterior or displays a system of transcuticular channels that open at the surface as minute slits of approximately 100 nm.

**Class-2 cells** are wedged in between class-1 cells and rest on the basement membrane but do not reach the cuticle (Box 1 Fig., blue). Secretion has to be released through the neighbouring class-1 cells. Class-2 gland cells are commonly found in termites (COSTA-LEONARDO & al. 2023) but are scarce or absent in other insects. In a follow-up study to their original gland classification, NOIROT & QUENNEDEY (1991) questioned the true glandular nature of class-2 cells and homologized them with oenocytes.

**Class-3 cells** are invariably connected with duct cells and therefore form part of bicellular units. Their complex ontogenesis goes through a process in which a stem cell forms a tetrad following two mitotic divisions, after which two cells degenerate and the remaining two cells differentiate into the secretory cell and its accompanying duct cell (SRENG & QUENNEDEY 1976). The junction of both cells is formed by the end apparatus, which acts as a draining device that collects secretion from the gland cell and guides it through the duct cell to the exterior (Box 1 Fig., red). The end apparatus is formed by a fenestrated cuticular canal surrounded by surface-increasing microvilli, which facilitate the release of the secretory products. The ducts usually have a constant diameter of 0.5 - 1  $\mu\text{m}$ , regardless the size of the insect, which is most likely determined by physiochemical properties of transportation of liquids.



**Box 1 Fig.:** Schematical survey of glandular differentiation of insect integument showing gland cells of classes 1, 2, and 3. Note size difference of gland pores of class-3 cells (gp, diameter usually 0.5 - 1  $\mu\text{m}$ ) and minute slits that may occur in the cuticle overlaying class-1 cells (ms, diameter in nanometer range). bi: basal invaginations, ct: cuticle, ea: end apparatus, mv: microvilli, nf: nerve fiber, S: sensillar cell, sp: sensillar pore.

**Tab. 1:** Overview of exocrine gland variety inside the mandibles of ants. The numbering corresponds with that used in this review and reflects the chronological order in which gland descriptions were published. Class refers to gland classification.

Nr	Gland	Occurrence	Class	Reference
1	dispersed intramandibular gland cells	all subfamilies except Agroecomyrmecinae	3	SCHOETERS & BILLEN (1994)
2	apicomesal intramandibular gland	<i>Strumigenys membranifera</i>	1	BILLEN & ESPADALER (2002)
3	ventral intramandibular gland	<i>Protanilla wallacei</i>	1	BILLEN & al. (2013), RICHTER & al. (2021b)
4	basimandibular sieveplate gland	<i>Tatuidris tatusia</i>	3	BILLEN & DELSINNE (2014)
5	mandibular pit gland	<i>Brachyponera</i> , <i>Cryptopone</i> , <i>Euponera</i>	1	BILLEN & AL-KHALIFA (2016)
6	intramandibular silk gland	<i>Rhopalomastix johorensis</i>	3	BILLEN & PEETERS (2020)
7	apicomandibular gland	<i>Strumigenys mutica</i> queens	3	WANG & al. (2021c)
8	ventral basimandibular gland	<i>Strumigenys</i>	1	BOLTON (1999), WANG & al. (2021c)
9	dorsoproximal intramandibular gland	<i>Leptanilla clypeata</i>	1	BILLEN & ITO (2022)
10	ventrolateral intramandibular gland	<i>Proceratium japonicum</i>	1	this review
11	distal mandibular blade gland	Pseudomyrmecinae, <i>Temnothorax ravouxi</i>	1	this review
12	contentious interpretations in literature	Attini	-	DO AMARAL & CAETANO (2006), MARTINS & SERRÃO (2011), MARTINS & al. (2015a)

**Tab. 2:** Survey of species with collection locality and number of specimens studied in this review. H, S, and T indicate that ants were studied with histology (H), scanning electron microscopy (S), or transmission electron microscopy (T). \* *P. wallacei* TAYLOR, 2013 nec HÖLLDOBLER & WILSON 1990 (BILLEN & al. 2013).

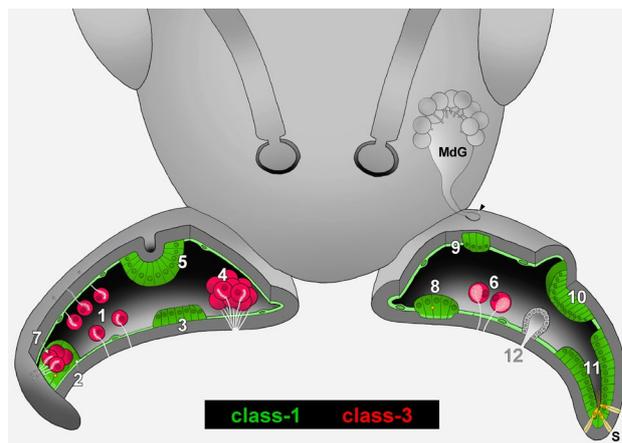
Species	Collection locality	Worker	Queen	Male
<b>Agroecomyrmecinae</b>				
<i>Tatuidris tatusia</i> BROWN & KEMPF, 1968	Copalinga Private Reserve, Ecuador	2 HST	0	0
<b>Amblyoponinae</b>				
<i>Amblyopone australis</i> ERICHSON, 1842	Nelligen, NSW, Australia	1 H	0	0
<i>Myopopone castanea</i> (SMITH, 1860)	Bac Giang Prov., Vietnam	1 H	0	0
<i>Prionopelta kraepelini</i> FOREL, 1905	Kebun Raya, Indonesia	2 H	0	0
<i>Stigmatomma roahady</i> ESTEVES & FISHER, 2016	Andrambovato, Madagascar	1 H	0	0
<b>Aneuretinae</b>				
<i>Aneuretus simoni</i> EMERY, 1913	Gilimale Forest, Sri Lanka	20 HST	0	0
<b>Dolichoderinae</b>				
<i>Liometopum microcephalum</i> (PANZER, 1798)	Debrecen, Hungary	2 H	0	0
<i>Tapinoma nigerrimum</i> (NYLANDER, 1856)	Ostend, Belgium	3 H	0	0
<b>Dorylinae</b>				
<i>Aenictus ceylonicus</i> (MAYR, 1866)	Nantou County, Taiwan	1 H	0	0
<i>Cerapachys sulcinodis</i> EMERY, 1889	Chiang Mai, Thailand	4 H	0	0
<i>Dorylus orientalis</i> WESTWOOD, 1835	Watinapaha, Sri Lanka	4 H	0	0
<i>Labidus praedator</i> (SMITH, 1858)	Manaus, AM, Brazil	2 HS	0	0
<i>Ooceraea biroii</i> (FOREL, 1907)	Kaohsiung, Taiwan	4 H	0	0
<b>Ectatomminae</b>				
<i>Heteroponera imbellis</i> (EMERY, 1895)	Misty Mountain, NSW, Australia	2 H	0	0
<i>Holcoponera strigata</i> (NORTON, 1868)	Chiapas, Mexico	1 H	0	0
<b>Formicinae</b>				
<i>Camponotus japonicus</i> MAYR, 1866	Yangling, SA, China	6 H	0	0

Species	Collection locality	Worker	Queen	Male
<i>Formica rufa</i> LINNAEUS, 1761	Ostend, Belgium	3 H	0	0
<i>Myrmoteras iriodum</i> MOFFETT, 1985	Ulu Gombak, Malaysia	5 HST	0	0
<i>Oecophylla longinoda</i> (LATREILLE, 1802)	Yaoundé, Cameroon	8 HST	2 H	0
<b>Leptanillinae</b>				
<i>Leptanilla clypeata</i> YAMANE & ITO, 2001	Bogor Gardens, Indonesia	8 HST	0	0
<i>Protanilla lini</i> TERAYAMA, 2009	Nantou County, Taiwan	6 HS	0	0
<i>Protanilla wallacei</i> TAYLOR, 2013 nec HÖLLDOBLER & WILSON, 1990*	Ulu Gombak, Malaysia	4 HST	3 HST	0
<b>Myrmeciinae</b>				
<i>Myrmecia nigriceps</i> MAYR, 1862	Sydney, NSW, Australia	2 HS	0	0
<i>Myrmecia pilosula</i> SMITH, 1858	Hobart, TAS, Australia	0	0	1 H
<i>Nothomyrmecia macrops</i> CLARK, 1934	Poochera, SA, Australia	2 HS	0	0
<b>Myrmicinae</b>				
<i>Atta laevigata</i> (SMITH, 1858)	Caracas, Venezuela	4 H	0	0
<i>Atta sexdens</i> (LINNAEUS, 1758)	Ribeirão Preto, SP, Brazil	4 HST	0	0
<i>Eurhopalothrix procera</i> (EMERY, 1897)	Pintung County, Taiwan	6 H	0	0
<i>Monomorium pharaonis</i> (LINNAEUS, 1758)	Leuven, Belgium	9 HT	1 HT	0
<i>Octostruma inca</i> BROWN & KEMPF, 1960	Copalinga Private Reserve, Ecuador	4 HT	0	0
<i>Rhopalomastix johorensis</i> WHEELER, 1929	Cha-Om, Thailand	6 HT	0	0
<i>Solenopsis geminata</i> (FABRICIUS, 1804)	Taichung, Taiwan	5 H	0	0
<i>Strumigenys circothrix</i> (OGATA & ONOYAMA, 1998)	Okinawa-Jima, Japan	0	0	2 H
<i>Strumigenys hexamera</i> (BROWN, 1958)	Takamatsu, Japan	5 H	0	0
<i>Strumigenys lacunosa</i> LIN & WU, 1996	Nantou County, Taiwan	2 H	1 H	0
<i>Strumigenys leptothrix</i> WHEELER, 1929	Nantou County, Taiwan	4 HS	0	0
<i>Strumigenys membranifera</i> EMERY, 1869	Barcelona, Spain	4 HST	0	0
<i>Strumigenys minutula</i> TERAYAMA & KUBOTA, 1989	Pingtung County, Taiwan	2 HT	0	0
<i>Strumigenys mutica</i> (BROWN, 1949)	Nantou County, Taiwan	8 HST	5 HST	0
<i>Strumigenys sauteri</i> (FOREL, 1912)	Nantou County, Taiwan	2 H	2 H	0
<i>Strumigenys solifontis</i> BROWN, 1949	Nantou County, Taiwan	6 HS	0	0
<i>Talaridris mandibularis</i> WEBER, 1941	Amazonas, Brazil	3 H	0	0
<i>Temnothorax ravouxi</i> (ANDRÉ, 1896)	Darmstadt, Germany	8 H	0	0
<b>Paraponerinae</b>				
<i>Paraponera clavata</i> (FABRICIUS, 1775)	Belem, PA, Brazil	2 HST	0	0
<b>Ponerinae</b>				
<i>Brachyponera chinensis</i> (EMERY, 1895)	Nantou County, Taiwan	7 H	0	0
<i>Brachyponera luteipes</i> (MAYR, 1862)	Changhua, Taiwan	3 H	0	0
<i>Brachyponera sennaarensis</i> (MAYR, 1862)	Riyadh, Saudi Arabia	8 HST	8 HST	2 H
<i>Cryptopone taivanae</i> (FOREL, 1913)	Nantou County, Taiwan	4 HST	0	0
<i>Euponera sharpi</i> FOREL, 1901	Changhua, Taiwan	2 H	0	0
<i>Myopias emeryi</i> (FOREL, 1913)	Ulu Gombak, Malaysia	3 H	0	0
<i>Myopias hollandi</i> (FOREL, 1901)	Padang, Indonesia	1 H	0	0
<b>Proceratiinae</b>				
<i>Discothyrea sauteri</i> FOREL, 1912	Nantou County, Taiwan	3 H	0	0
<i>Probolomyrmex longinodus</i> TERAYAMA & OGATA, 1988	Okinawa-Jima, Japan	2 H	0	0
<i>Proceratium japonicum</i> SANTSCHI, 1937	Nantou County, Taiwan	6 HST	2 H	0
<b>Pseudomyrmecinae</b>				
<i>Pseudomyrmex oculatus</i> (SMITH, 1855)	Rio Preto, SP, Brazil	3 H	0	0
<i>Pseudomyrmex schuppi</i> (FOREL, 1901)	Piracicaba, SP, Brazil	7 HT	0	0
<i>Tetraponera allaborans</i> (WALKER, 1859)	Nantou County, Taiwan	6 H	0	0
<i>Tetraponera rufonigra</i> (JERDON, 1851)	Bangkok, Thailand	4 HST	0	0

The main tissue inside the mandibles is formed by the integumental epidermis, nerve fibres, and sensilla, together with various exocrine glands.

The major exocrine structure associated with the mandibles is the paired mandibular gland with functions ranging from nestmate alarm to antimicrobial defence (MASCHWITZ 1964, HOENIGSBERGER & al. 2020). The slit-shaped orifice of this gland is located at the upper base of the mandible on an area known as the mandalus. Its closing mechanism is remarkably similar across species as it depends on the retraction of the prepharyngeal sucking pump that is connected via an anchor-shaped continuation of the mandibular gland duct (Fig. 1F) to the base of the mandalus (RICHTER & al. 2021a). The major part of the mandibular gland, however, is entirely located inside the head capsule, and in exceptional cases may even extend into the thorax and abdomen (*Colobopsis*: DAVIDSON & al. 2012). The rounded to polygonal secretory cells belong to class-3 (for gland classification, see Box 1) and are connected via accompanying duct cells to a thin-walled reservoir sac (Fig. 1D). In some cases, the cluster of secretory cells can be surrounded by a protective sheath of connective tissue (Fig. 1E), as was observed in *Brachyponera sennaarensis* (see BILLEN & AL-KHALIFA 2018) and *Myrmoteras iriodum* (see BILLEN & al. 2016). The ultrastructure of the secretory cells usually contains a well-developed smooth endoplasmic reticulum, and numerous mitochondria and secretory vesicles (Fig. 1G). These cellular characteristics are in agreement with the pheromonal function of this gland (BILLEN & SCHOETERS 1994, BOONEN & al. 2013, XU & al. 2023). Another mandible-associated gland is formed by a cluster of secretory cells in *Eciton* queens, which opens through the intersegmental membrane that connects the head capsule with the mandible (HÖLLDOBLER 2016). Since the latter as well as the mandibular glands are not located inside the mandibles, several other exocrine glands that occur inside the mandibles are referred to as intramandibular glands.

In this review, we present a survey of 11 such intramandibular glands that include structures formed both by class-1 and others formed by class-3 secretory cells (Box 1). The description of these glands, as summarized in Figure 2 and Table 1, follows the chronological order in which they have been described in literature and includes a revision of the designation covering the various gland names. Our work is based on the examination of 58 species representing 14 of the 16 currently recognized ant subfamilies (BOLTON 2024). With only the extremely elusive Martialinae and Apomyrminae not available, this review covers the most extensive subfamily representation to date for a mainly histology-based study (Tab. 2). The methodology used in this review includes light and electron microscopy (transmission electron microscopy, TEM, and scanning electron microscopy, SEM). For light microscopy and TEM, the anterior part of the head including the mandibles of live ants was cut off and fixed in 2% glutaraldehyde in a Na-cacodylate buffer followed by



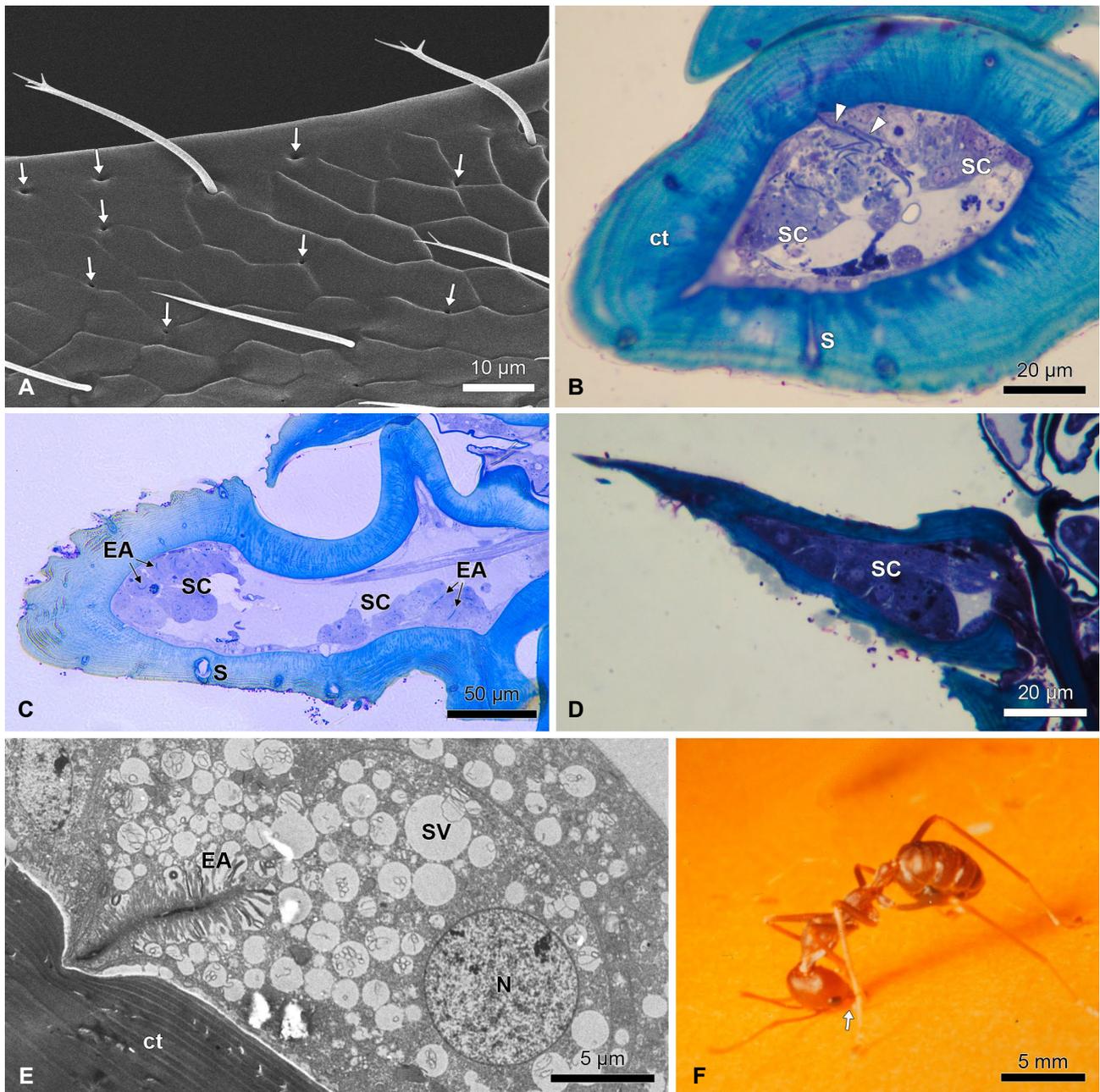
**Fig. 2:** Schematic illustration of the variety of exocrine glands inside ant mandibles: **1.** Dispersed intramandibular gland cells. **2.** Apicomasal intramandibular gland. **3.** Ventral intramandibular gland. **4.** Basimandibular sieveplate gland. **5.** Mandibular pit gland. **6.** Intramandibular silk gland. **7.** Apicomandibular gland. **8.** Ventral basimandibular gland. **9.** Dorsoproximal intramandibular gland. **10.** Ventrolateral intramandibular gland. **11:** Distal mandibular blade gland. Structure **12** has been wrongly interpreted as epithelial glands with reservoir but forms part of sensilla (S) (our drawing of this structure imitates its appearance as in MARTINS & SERRÃO 2011, MARTINS & al. 2015a). Yellow dots in 2 and 8 represent nerve fibers. The position of the mandibular gland (MdG) in the head capsule is illustrated at the right side. This gland opens through a slit-shaped mandalus at the upper mandibular base (arrowhead).

postfixation in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, tissues were embedded in araldite. Serial semithin sections of 1  $\mu$ m thickness for light microscopy were made with a Leica (Wetzlar, Germany) EM UC6 ultramicrotome. These were stained with methylene blue and thionin and viewed under an Olympus (Tokyo, Japan) BX-51 microscope. Sections for TEM with a thickness of 70 nm were double-stained with uranyl acetate and lead citrate and viewed under a Zeiss (Oberkochen, Germany) EM900 electron microscope. Heads for scanning microscopy were attached to aluminium stubs with double-adhesive tape, sputtered with gold, and examined under a JEOL (Akishima, Japan) JSM-6360 scanning microscope. In all figures with longitudinal orientation, the anterior is to the left.

## Overview of the various intramandibular glands

### 1. Dispersed intramandibular gland cells

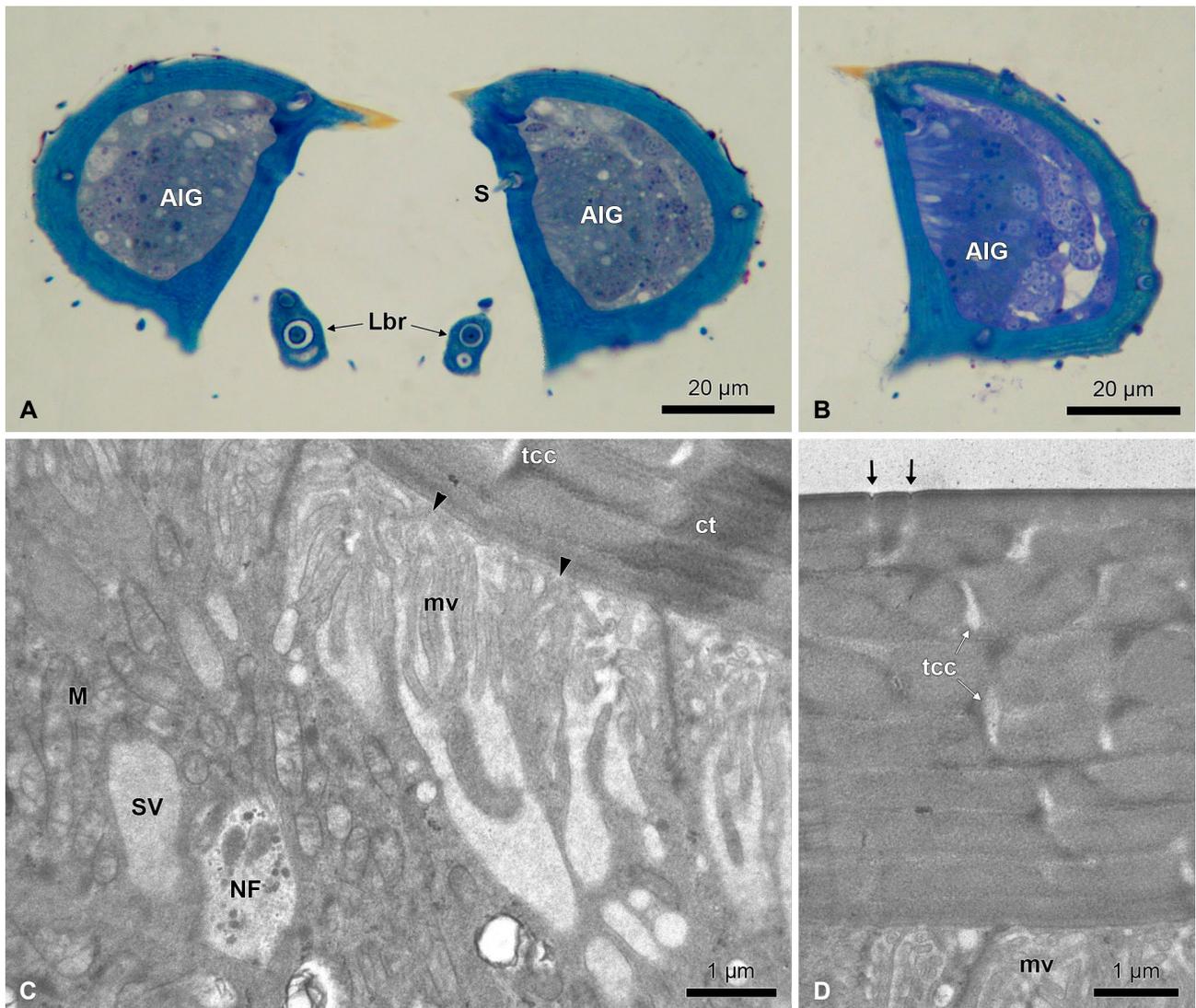
Following a brief preliminary report in leaf-cutting ants (TOLEDO 1967), the first large-scale discovery of exocrine tissue inside the mandibles of ants was reported by SCHOETERS & BILLEN (1994), who found dispersed class-3 cells in nine subfamilies (Fig. 3A - E). Although the histological descriptions mainly refer to workers,



**Fig. 3:** Dispersed intramandibular gland cells. (A) Scattered duct openings (arrows) on upper surface of mesal edge of mandible of *Strumigenys solifontis* worker. (B) Section through mandible of *Aenictus ceylonicus* worker showing secretory cells (SC) and duct cells (arrowheads). ct: cuticle, S: sensilla. (C) Secretory cells with end apparatus (EA) in *Eurhopalothrix procera* worker. (D) Mandible of *Strumigenys circothrix* male. (E) Secretory cell in *Myrmoteras iriodum* worker showing end apparatus and secretory vesicles (SV). N: nucleus. (F) Worker of *Oecophylla longinoda* rubbing the substrate with upper side of its mandibles (arrow).

similar gland cells were also found in males (Fig. 3D) and queens (SCHOETERS & BILLEN 1994). The dispersed intramandibular gland cells, which range from 15 - 20  $\mu\text{m}$  in diameter, usually occur as single units or in pairs, sometimes occurring in one or two rows along the mandible's masticatory margin (GRASSO & al. 2004). The accompanying ducts mainly open at the upper mandibular surface as small pores with a diameter of 0.5 - 1  $\mu\text{m}$  (Fig. 3A). Ultrastructural examination clearly shows the end apparatus (Fig. 3E) and reveals the presence of a well-developed smooth endoplasmic reticulum, which indicates the

cells produce a non-proteinaceous and therefore possibly pheromonal secretion (SCHOETERS & BILLEN 1994). Our present survey updates the occurrence of these gland cells in 13 of the 16 extant subfamilies. Among sampled subfamilial taxa, such gland cells were not detected only in Agroecomyrmecinae. In *Tatuidris tatusia*, one of the two extant species of this subfamily, however, a peculiar basimandibular sieveplate gland occurs, which may represent a modification of the dispersed gland cells by the clustered opening of the ducts (see further under "4. Basimandibular sieveplate gland").



**Fig. 4:** Apicomasal intramandibular gland (AIG) in *Strumigenys membranifera* worker. (A), (B) Transverse sections near mandible tip showing conspicuous gland epithelium. Lbr: labrum, S: sensilla. (C) Apical epithelium showing irregular microvilli (mv), transcuticular channels (tcc) and subcuticular cytoplasmic layer (arrowheads). Note presence of nerve fiber (NF). ct: cuticle, M: mitochondria, SV: secretory vesicles. (D) Detail of cuticle with transcuticular channels and their opening at the cuticular surface as minute slits (arrows).

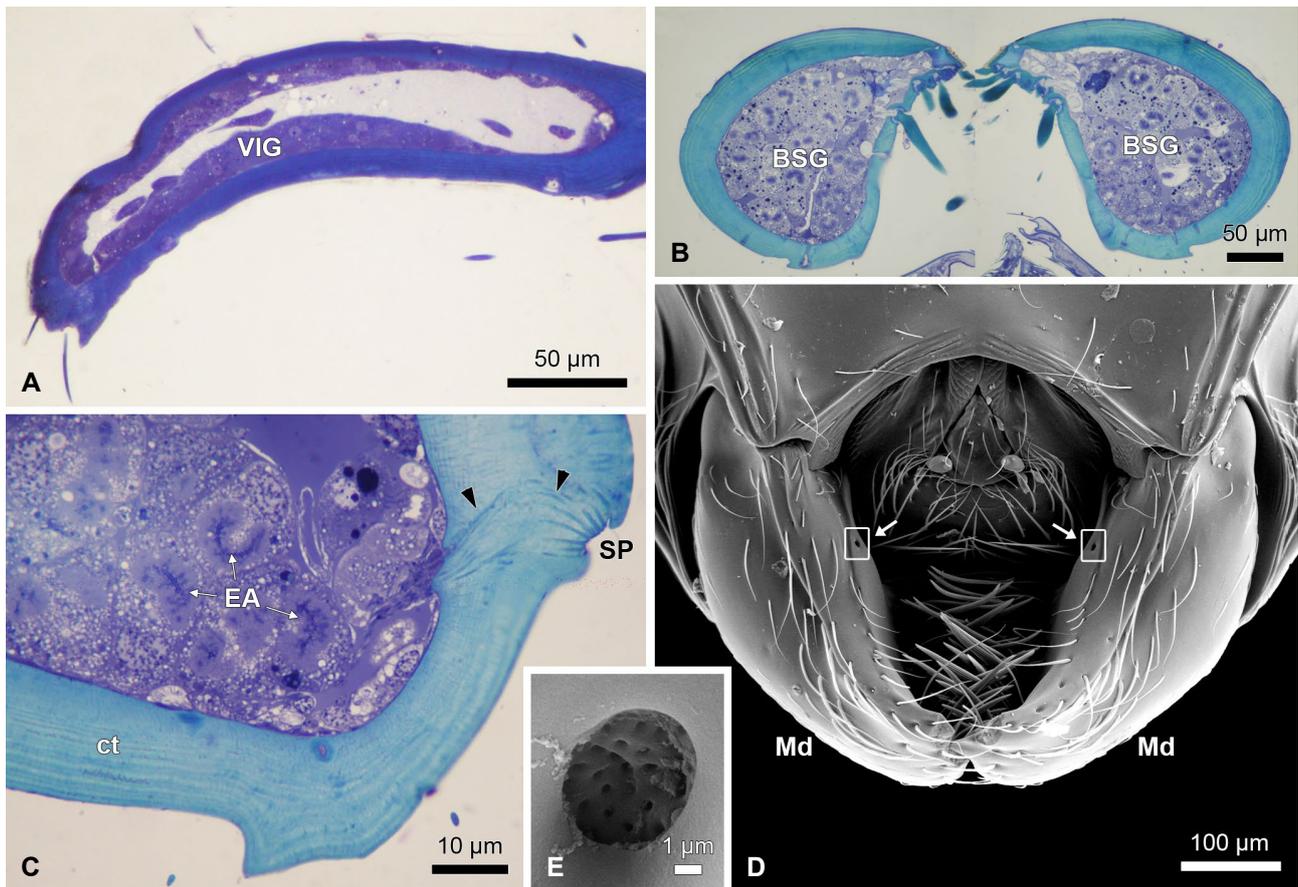
The function of the glandular secretion is not yet clear, although a behavioural study in *Oecophylla longinoda* revealed that this gland is used to mark the substrate, which results in recruitment of nestmates. This behaviour can be observed when workers discover a new food item, new territory, or an alien ant. In such situations, the ant first rubs the lower side of its mandibles against the substrate, followed by a peculiar head tilting and then rubbing with the upper side of the mandibles (Fig. 3F). Histological sections confirmed the occurrence of gland cells underneath both the upper and lower mandibular surface, which is in agreement with the observed rubbing behaviour (ROUX & al. 2010). Chemical analysis of this intramandibular gland of *Neoponera villosa* showed the presence of linear and branched alkanes (MARTINS & al. 2015b), although no clear function could be attributed to these (MARTINS & al. 2016). *Aneuretus simoni* also has gland cells underneath

the upper as well as lower side of the mandibles, although its cells are characterized by a well-developed rough endoplasmic reticulum. This finding indicates the production of a proteinaceous secretion and therefore makes it unlikely that it has a pheromonal function in this species (BILLEN & VERBESSELT 2016).

The occurrence of dispersed gland cells in the mandibles of other social insects was first reported for bumblebees (NEDEL 1960) and later was also confirmed for stingless bees (COSTA-LEONARDO 1978), wasps (PENAGOS-ARÉVALO & al. 2015), and termites (BERÁNKOVÁ & al. 2022).

## 2. Apicomasal intramandibular gland

BILLEN & ESPADALER (2002) described an “epithelial intramandibular gland” underneath the mesal mandibular tip in workers of *Strumigenys membranifera* (Fig. 4A, B).



**Fig. 5:** (A) Longitudinal section through mandible of *Protanilla lini* worker showing ventral intramandibular gland (VIG). (B) - (E) Basimandibular sieveplate gland (BSG) of *Tatuidris tatusia* worker. (B), (C) Transverse semithin sections through mandible filled with gland cells and opening of ducts (arrowheads) through sieveplate (SP). ct: cuticle, EA: end apparatus. (D) Ventral view scanning micrograph of mandibles, white frames show position of sieveplates. Md: mandible. (E) Enlargement of framed area in 5D showing sieveplate.

The gland is formed by conspicuous columnar epithelial cells with a height of 20  $\mu\text{m}$ , whereas the non-glandular integumental epidermis hardly measures 1 - 2  $\mu\text{m}$ . The cuticle that overlays the gland epithelium has a reduced thickness, which gives the gland a bowl-shape (Fig. 4A, B). Since several other epithelial glands have been described inside the mandibles, we suggest revising the name of this gland to the “apicomesal intramandibular gland”. The original description included the occurrence of apical microvilli (Fig. 4C) and transcucular channels that open at the cuticular surface as minute pores (Fig. 4D). Re-examination of this gland provided us with evidence of the irregular arrangement of the microvilli with the existence of a subcuticular cytoplasmic layer (arrowhead in Fig. 4C). It additionally revealed a conspicuous nerve fibre that is wedged between the epithelial cells (Fig. 4C).

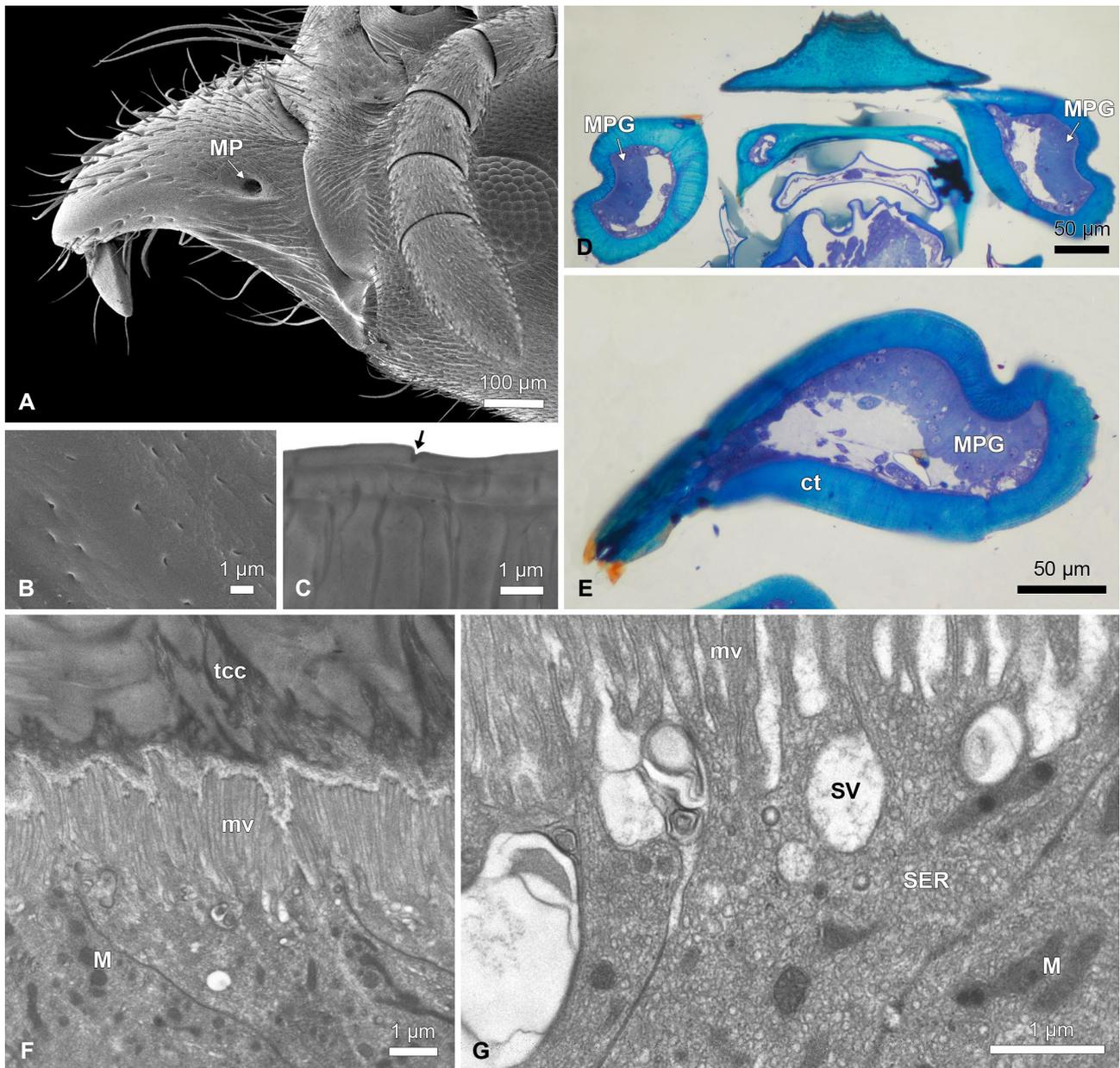
The presence of a subcuticular cytoplasmic layer and a nerve fibre are characteristics that were also found in the ventral basimandibular gland, which is exclusive for *Strumigenys* ants (WANG & al. 2021c; see also “8. Ventral basimandibular gland”). The location of both glands is clearly different, however, as the apicomesal intramandibular gland is found near the mesodistal tip of the mandible,

whereas the ventral basimandibular gland has a ventro-proximal location. Careful examination of *Strumigenys membranifera* moreover revealed the occurrence of both glands.

The function of the apicomesal intramandibular gland remains unknown. It was suggested by BILLEN & ESPADALER (2002) that it could possibly play a role in the attraction of Collembola prey (DEJEAN 1985), although this is not very likely as we did not find this gland in other *Strumigenys* species.

### 3. Ventral intramandibular gland

A prominently thickened epithelium with a thickness of 20 - 30  $\mu\text{m}$  was reported along the ventral mandibular wall in workers of *Protanilla lini* (“mandible internal gland”: RICHTER & al. 2021b) and in workers and queens of *Protanilla wallacei* (“intramandibular epithelial gland”: BILLEN & al. 2013; Fig. 5A). We suggest this gland be designated as the “ventral intramandibular gland”. We did not have sufficient available material for ultrastructural examination. The ventral location in the proximity of the site of food intake indicates a possible digestive function (BILLEN & al. 2013), but this needs to be verified. The gland



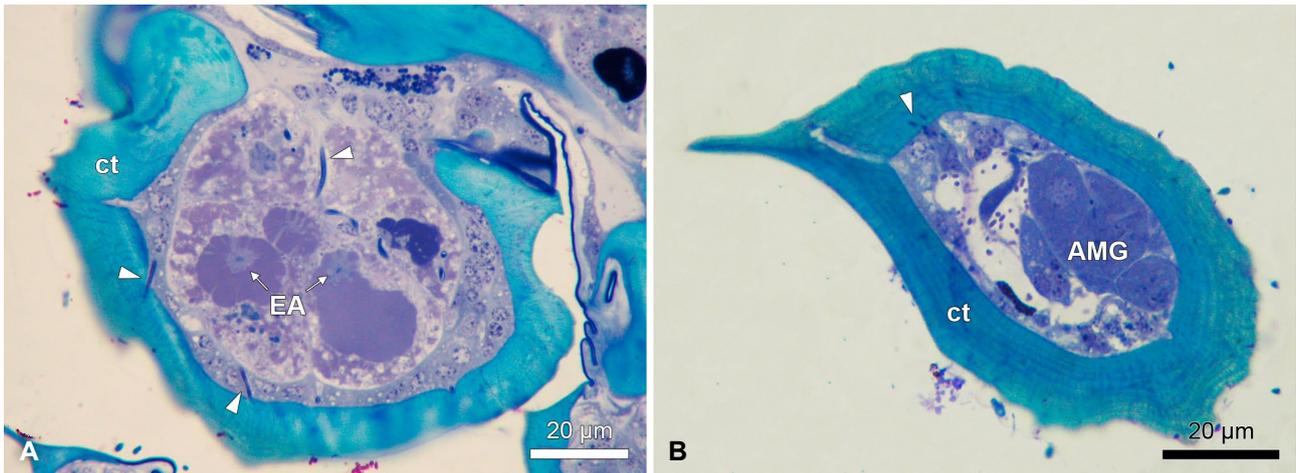
**Fig. 6:** (A) Profile view of anterior head part of *Brachyponera sennaarensis* worker with indication of mandibular pit (MP). (B) Detail of upper mandibular surface near pit showing numerous minute slits (*B. sennaarensis* worker). (C) Detail of cuticular surface at pit bottom, arrow indicates opening of minute slit (*Cryptopone taiwanae* worker). (D) Transverse section through proximal part of mandibles of *Brachyponera luteipes* worker showing conspicuous epithelium of mandibular pit gland (MPG). (E) Longitudinal section through mandible with MPG of *Euponera sharpi* worker. ct: cuticle. (F), (G) Apical part of MPG epithelium of *B. sennaarensis* worker showing transcuticular channels (tcc), regular microvilli (mv) and abundant smooth endoplasmic reticulum (SER). M: mitochondria, SV: secretory vesicles.

so far has only been found in *Protanilla*, although a gland with a similar location was also reported in some stingless bees (COSTA-LEONARDO 1978).

#### 4. Basimandibular sieveplate gland

The mandibles in workers of the agroecomyrmecine species *Tatuidris tatusia* are filled up with spherical class-3 cells with a diameter of 25 - 30 µm (BILLEN & DELSINNE 2014; Fig. 5B). Their ducts converge to a circular sieveplate (Fig. 5C, E) at the ventroproximal region of the

mandibles (Fig. 5D, E). Based on this anatomical appearance, we suggest naming it the “basimandibular sieveplate gland”. Each sieveplate contains 20 - 25 pores with a diameter of 0.5 µm, also indicating the number of secretory cells. Material for ultrastructural study was not available. The function of this gland remains unknown. *Tatuidris* is probably a specialist top predator on other invertebrates of the leaf-litter food web (BROWN & KEMPF 1968, JACQUEMIN & al. 2014). We speculate that the slow-moving workers may attract prey with chemicals produced by this or other



**Fig. 7:** (A) Transverse section through proximal part of mandible of *Rhopalomastix johorensis* worker showing cells of intramandibular silk gland with enlarged end apparatus (EA) and ducts crossing cuticle (arrowheads). ct: cuticle. (B) Transverse section through mandible tip of *Strumigenys mutica* queen showing apicomandibular gland (AMG) and ducts (arrowhead) crossing cuticle.

glands, although such behaviour can only be studied when live colonies of this elusive species will become available in future.

### 5. Mandibular pit gland

Among the Ponerinae, *Hagensia* are characterized by a conspicuous dorsoproximal groove in their mandibles, while *Brachyponera*, *Cryptopone*, and *Euponera* have a mandibular pit at the same position (Fig. 6A; SCHMIDT & SHATTUCK 2014). Histological examination of species of the latter three genera revealed this pit is associated with a 40 - 45 µm-thick glandular epithelium that extends along the lateral walls of the mandible (Fig. 6D, E). The mandibular surface that overlays the glandular epithelium displays numerous minute slits of 100 nm (Fig. 6B). Similar slits also occur along the external surface of the pit (Fig. 6C). Ultrastructural examination reveals the presence of transcuticular channels that connect to these slits, regularly arranged apical microvilli, and abundant smooth endoplasmic reticulum (Fig. 6F, G). This cytoplasmic composition of the mandibular pit gland is indicative for the production of a non-proteinaceous and hence possibly pheromonal secretion (BILLEN & AL-KHALIFA 2016). The pit may allow temporary storage of secretion, similar to an evaporatorium, although its broad connection to the exterior contradicts a proper reservoir function. The anatomical appearance of the mandibular pit makes it a challenge to study the chemical composition of the glandular secretion. Using microcapillaries on live ants may hopefully shed light on the function of this gland.

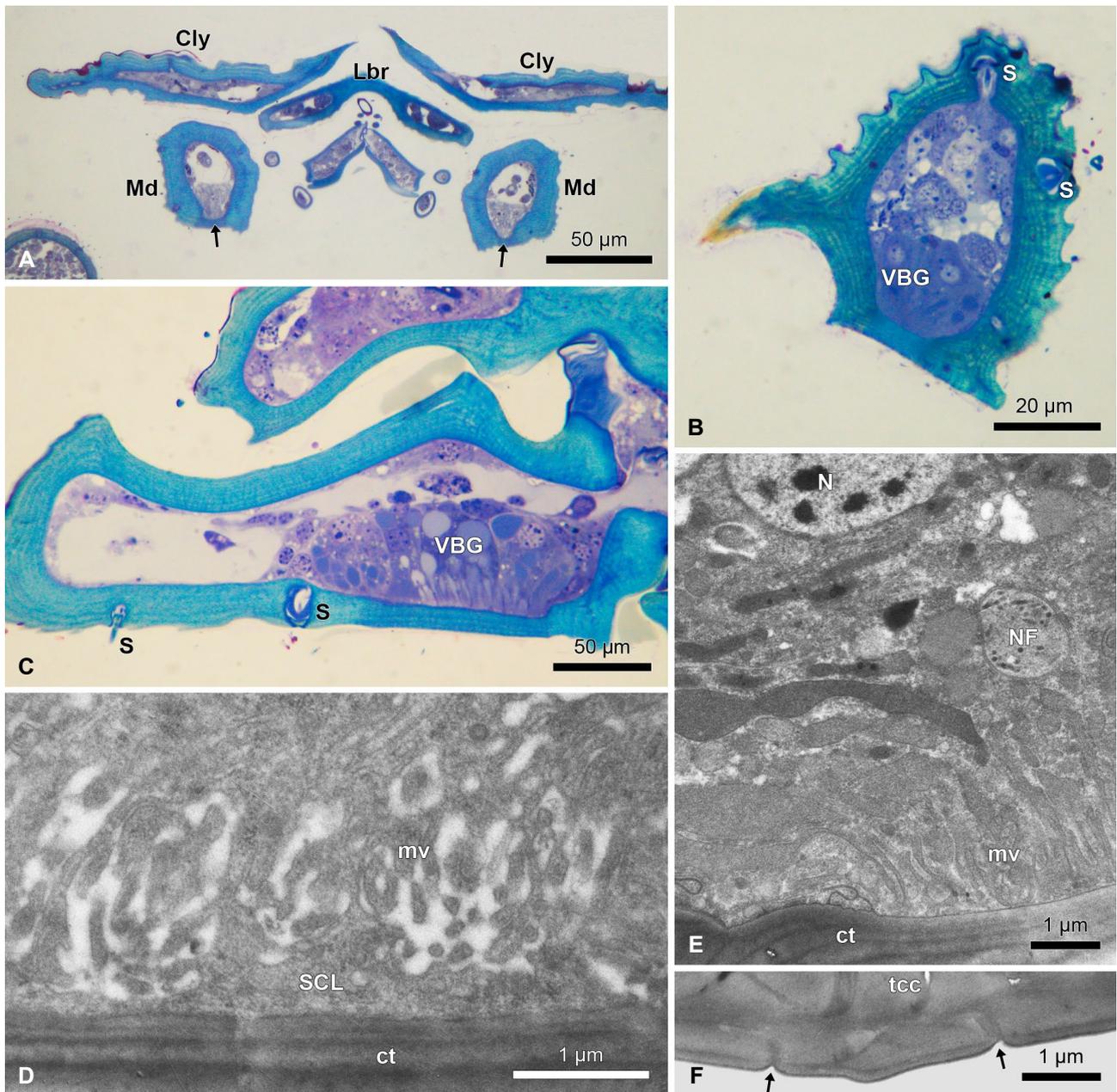
### 6. Intramandibular silk gland

In their report on silk production in *Melissotarsus* and *Rhopalomastix* ants, BILLEN & PEETERS (2020) described the occurrence of numerous large secretory cells in the anterior ventral portion of the head and their opening

through grooves along the semicircular margin of the buccal cavity. Besides these gland cells in the head capsule, *Rhopalomastix* workers also contain approximately 10 secretory cells inside the mandibles (BILLEN & PEETERS 2020). The ducts of these cells do not open through the buccal cavity grooves as is the case for the cells in the head capsule but directly cross the mandibular cuticle and therefore represent intramandibular silk gland cells (Fig. 7A). The cells have a diameter of around 30 µm and are characterized by a conspicuously enlarged end apparatus. As the appearance of these intramandibular cells in *Rhopalomastix* is similar to that of the cells in the head capsule, it is tempting to consider both as silk-producing glands. Species of both genera establish their nests in live wood under the bark of healthy trees in which they chew tunnels and galleries. They cover the tunnel walls with silk into which they anchor wood frass to ensure a better protection (BILLEN & PEETERS 2020).

### 7. Apicomandibular gland

A recent study of the mandibles in 22 *Strumigenys* species revealed the existence of a cluster of class-3 gland cells at the tip of the mandibles that only occurs in queens of *Strumigenys mutica* (WANG & al. 2021c). This apicomandibular gland is formed by approx. 10 round cells with a diameter of 12 µm whose ducts open through the cuticle at the inner margin of the mandibular tip (Fig. 7B). The restricted presence of this gland to queens of *S. mutica* only possibly can be interpreted by the social parasitic lifestyle of this species. After her nuptial flight, the young *S. mutica* queen penetrates a colony of a host species (such as *Strumigenys solifontis*) and kills the resident queen (WANG & al. 2021c, MIZUNO & al. 2025). No further information is available on this usurpation, although the exclusive presence of this novel apicomandibular gland in *S. mutica* queens, as far as known, suggests a possible role in this process.

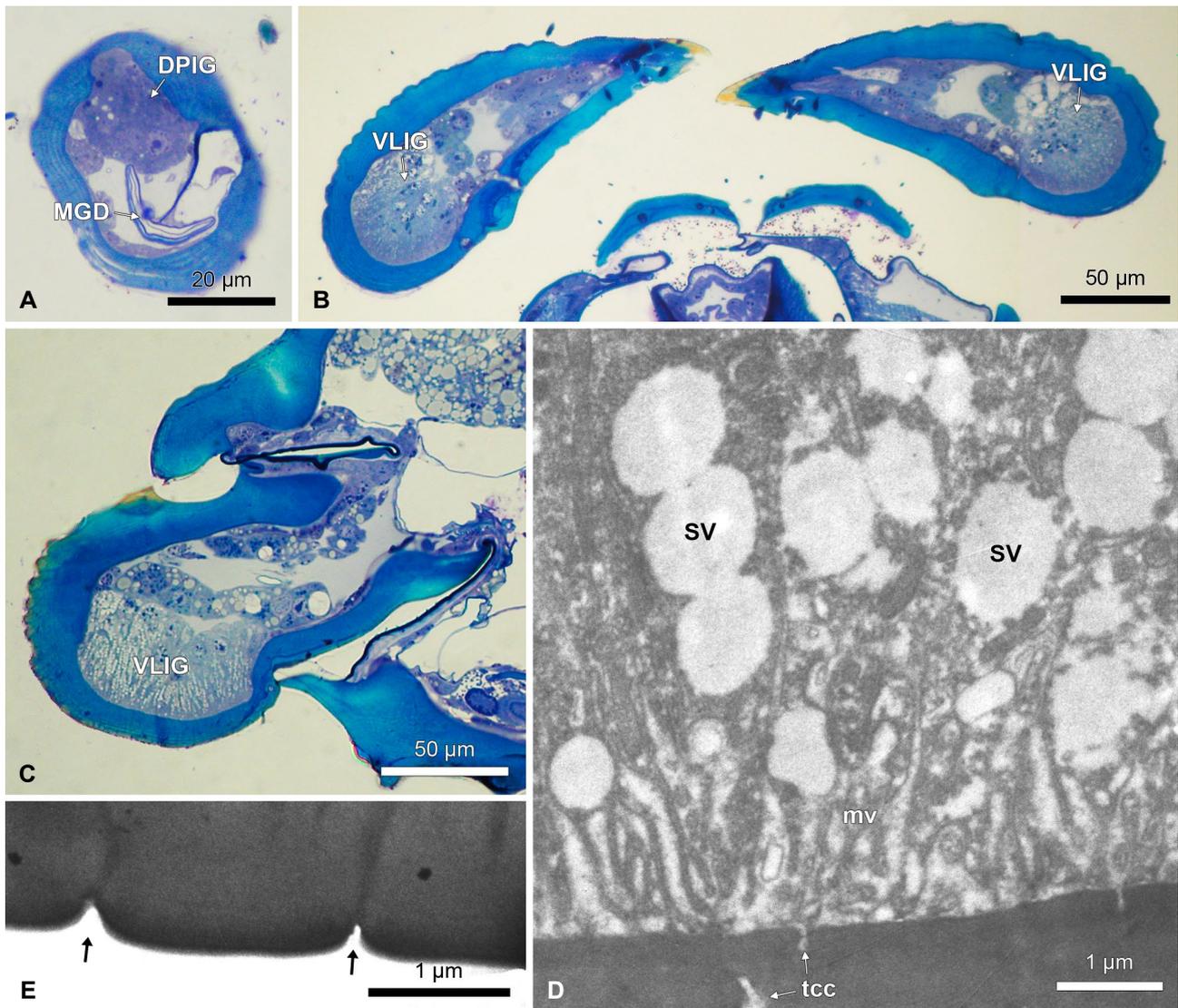


**Fig. 8:** Ventral basimandibular gland (VBG) exclusive for *Strumigenys* ants. (A) Transverse section through anterior head tip of *Strumigenys canina* worker showing position of VBG (arrows). Cly: clypeus, Lbr: labrum, Md: mandibles. (B) Transverse section through proximal part of mandible of *Strumigenys hexamera* worker; S: sensilla. (C) Longitudinal section through mandible of *Strumigenys membranifera* worker. (D) Detail of apical cytoplasm of *Strumigenys minutula* worker showing irregular microvilli (mv) and subcuticular cytoplasmic layer (SCL). ct: cuticle. (E) VBG epithelium of *Strumigenys lacunosa* worker, note presence of nerve fiber (NF). N: nucleus. (F) Detail of cuticle surface of *Strumigenys leptothrix* worker showing opening of minute slits (arrows). tcc: transcuticular channels.

### 8. Ventral basimandibular gland

*Strumigenys* ants are particularly interesting in the study of exocrine glands as they possess four glands that are exclusive for the genus (BOLTON 1999: basimandibular and ventral scape glands in the head, apicofemoral and apicotibial glands in the legs; the presumed “mesopleural gland”, however, does not represent glandular tissue: WANG & al. 2021b).

The ventral basimandibular gland in workers and queens of all examined species occurs underneath the ventroproximal cuticle of the mandible as an epithelium formed by class-1 cells with a thickness of 15 - 25  $\mu$ m (Fig. 8A - C). Preliminary observation of males also indicates the presence of a thickened epithelium. As the cuticle in the region that overlays the gland epithelium is only half as thick compared with that in the non-glandular part, the



**Fig. 9:** (A) Transverse section through proximal part of mandible of *Leptanilla clypeata* worker showing dorsoproximal intramandibular gland (DPIG). MGD: mandibular gland duct. (B) - (E) Ventrolateral intramandibular gland (VLIG) of *Proceratium japonicum* worker. (B) Transverse and (C) Longitudinal section through mandibles showing VLIG. (D) Detail of external layer of cuticle, arrows indicate minute slits. (E) Apical part of epithelium showing transcuticular channels (tcc), microvilli (mv), and secretory vesicles (SV).

gland has a bowl-shaped appearance (WANG & al. 2021b). Electron microscopy shows an irregular arrangement of apical microvilli that are separated from the cuticle by a thin subcuticular cytoplasmic layer (Fig. 8D). The epithelial cells contain smooth endoplasmic reticulum, which corresponds with the production of a non-proteinaceous and therefore possibly pheromonal secretion (WANG & al. 2021c). Another conspicuous ultrastructural characteristic is the presence of one or two swollen nerve fibres that are wedged in between the epithelial cells. They have a diameter of 1.5  $\mu\text{m}$  and are loaded with dark neurosecretory vesicles of 50 - 80 nm (Fig. 8E). These three features, namely a bowl shape, presence of a subcuticular cytoplasmic layer on top of the microvilli, and a nerve fiber wedged between the epithelial cells, represent an exclusive characteristic of class-1 integumental glands in *Strumigenys* (ventral basi-

mandibular gland: WANG & al. 2021c; ventral scape gland: WANG & al. 2021a; apicofemoral and apicotibial glands: WANG & al. 2023, see also “2. Apicomesal intramandibular gland”). The cuticle covering the glandular epithelium contains irregular transcuticular channels that open to the exterior through minute slits of approximately 100 nm (Fig. 8F). Similar slits on the cuticular surface overlaying epithelial glands were also reported for the apicofemoral and apicotibial glands in *Strumigenys* (WANG & al. 2023) and the mandibular pit gland in some Ponerinae (see also “5. Mandibular pit gland”).

### 9. Dorsoproximal intramandibular gland

Workers of *Leptanilla clypeata* have a large class-1 gland underneath an obvious excavation of the dorso-proximal cuticle (Fig. 9A). The epithelium has a thickness

of 15 µm and is formed by columnar cells with basally located round nuclei. Ultrastructural examination shows apical microvilli that reach the cuticle and deep basal invaginations (BILLEN & ITO 2022). The cytoplasm contains smooth endoplasmic reticulum, a Golgi apparatus, and numerous mitochondria. These organelles indicate a possible production of pheromonal secretion, although the function of the gland remains unknown. The surface of the cuticle that overlies the gland epithelium is smooth, and at high magnification, minute pores of 100 nm are clearly visible (BILLEN & ITO 2022).

#### 10. Ventrolateral intramandibular gland

A novel gland that we describe here was found in *Proceratium japonicum* workers and queens. The gland is located along the ventrolateral outer margin of the mandibles and is formed by a prominent epithelium of columnar class-1 cells with a thickness of 40 µm (Fig. 9B, C). The cuticle shows transcuticular channels that open at the mandibular surface through minute slits of 100 nm (Fig. 9D). The apical region of the cells contains an abundance of round electron-lucent secretory vesicles with a diameter of 1 µm and a layer of fairly regular microvilli that reach the cuticle (Fig. 9E). The combination of its location and histological appearance together with its ultrastructural characteristics distinguish this ventrolateral intramandibular gland from other glands in the mandibles and support its designation as a novel exocrine gland. We did not find this gland in the other Proceratiinae that were examined (*Discothyrea sauteri* and *Probolomyrmex longinodis*).

#### 11. Distal mandibular blade gland

We found another obvious epithelial thickening along the distal part of the mandibular blade, which is the region with the masticatory margin. We observed this gland in workers of Pseudomyrmecinae (*Pseudomyrmex oculatus*, *Pseudomyrmex schuppi*, *Tetraoponera allaborans*, and *Tetraoponera rufonigra*). It consists of tall class-1 cells with a height of 30 - 40 µm that are found along both the dorsal and ventral part of the mandibular blade (Fig. 10A, B). We could not examine the ultrastructural characteristics of this gland, except for an observation of the mandibular surface with scanning microscopy that reveals the occurrence of numerous minute slits of 100 nm (Fig. 10A inset). The conspicuous size of the epithelium and the presence of minute slits on the cuticular surface that overlies the thickened epithelium support the interpretation of the glandular nature of the epithelium. Histological examination shows that the epithelium, especially at the masticatory margin, is interrupted by several sensillar cells of which some connect with sensory hairs (Fig. 10A, B). Glandular thickening of the epidermal lining of the entire mandibular wall has been described for social bees (COSTA-LEONARDO 1978) and wasps (PENAGOS-ARÉVALO & al. 2015: "intramandibular gland I"); thus, it is considerably larger than the blade region we describe.

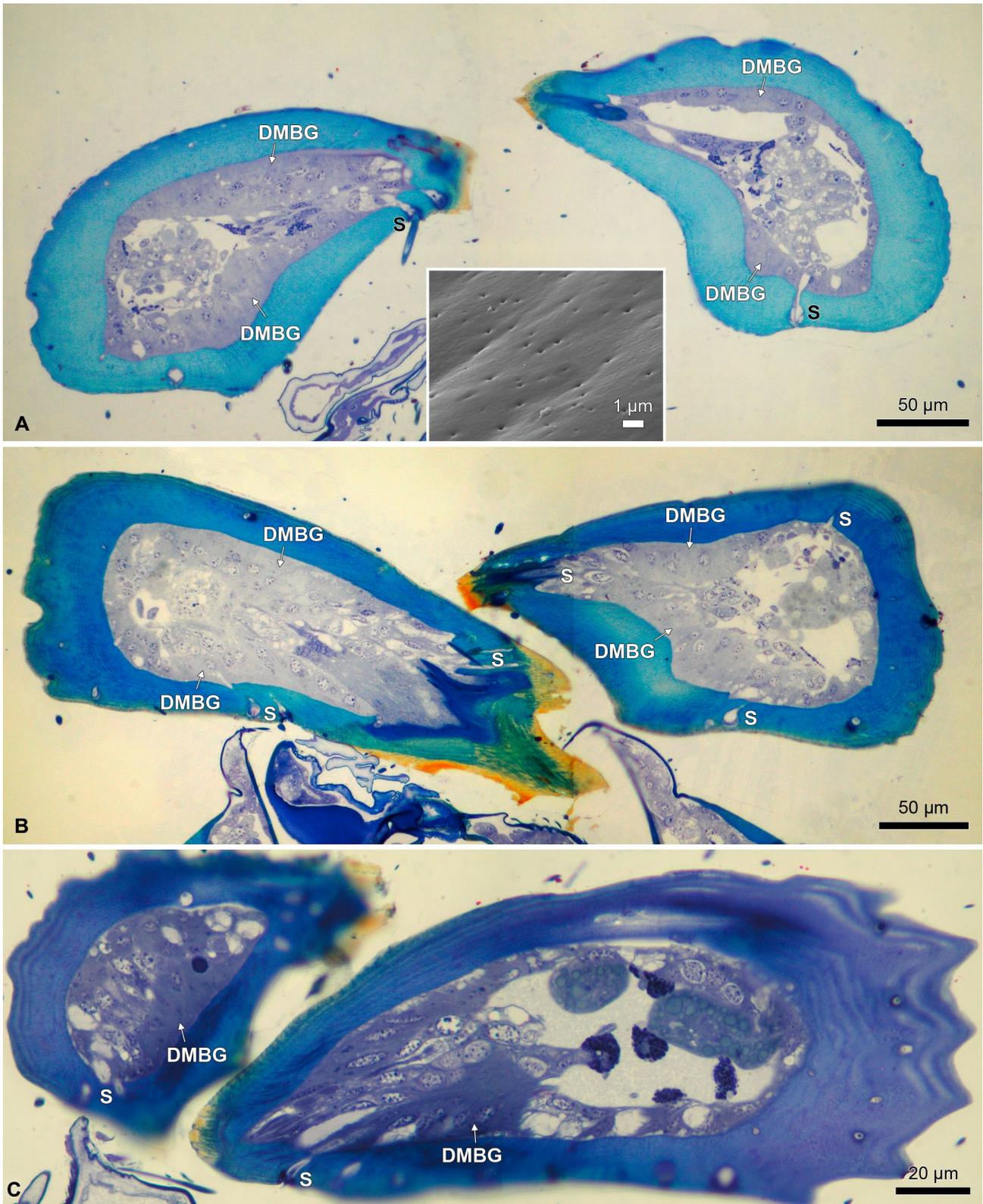
A comparable thickening of the integumental epithelium of the mandibular blade was also found in *Temno-*

*thorax ravouxi* workers (formerly *Epimyrma goesswaldi*). Numerous sensilla are wedged in between the epithelial cells at the masticatory margin (Fig. 10C). *Temnothorax ravouxi* is a slave-making myrmecine ant that organizes raids to steal the pupae from colonies of a host species and retrieve these to their nest where they eclose as slave workers (WINTER 1979). During colony foundation, the *T. ravouxi* queen penetrates a host species colony where she kills the host queen by slowly throttling her with the mandibles (BUSCHINGER 1986). This fighting behaviour raised the question whether *T. ravouxi* has a glandular weapon in its mandibles (A. Buschinger, pers. comm.), although it remains to be confirmed whether the distal mandibular blade gland and / or its associated sensilla may play an active role in this regard.

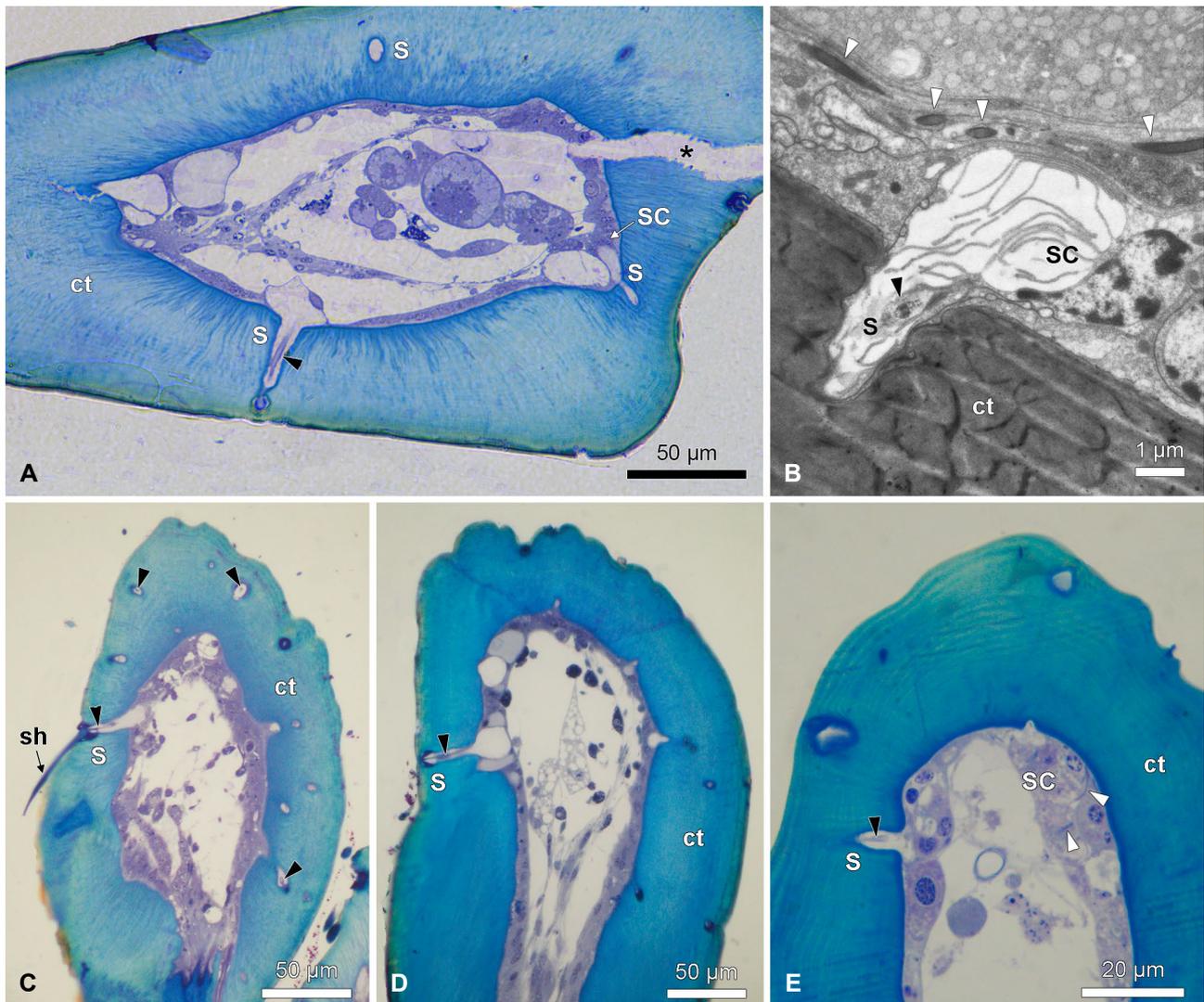
#### 12. Contentious interpretations in literature

In a study of the intramandibular glands of *Atta sexdens rubropilosa*, DO AMARAL & CAETANO (2006) described the presence of a secretory epithelium that contains "reservoirs" although the figures that should support this claim indicated sensilla. This contentious interpretation was repeated in two other papers that also failed to provide convincing histological evidence of the existence of such epithelial reservoirs (MARTINS & SERRÃO 2011, MARTINS & al. 2015a). Moreover, these publications include misleading drawings that suggestively show epithelium-lined reservoir sacs in a drawing style that we intentionally imitated in structure #12 in our Figure 2. We carefully examined workers of *Atta laevigata* in order to check the same species studied in MARTINS & al. (2015a) but could only find multiple sensilla instead of any glandular reservoir-like structures (Fig. 11A).

Sensilla are common constituents of insect mandibles, they presumably evolved from epidermal hairs (Fig. 11A - E). The most common type I sensilla can be defined as sense organs containing a specialized sensory cuticle associated with one or more bipolar neurons of which the dendrites are enveloped by at least two sheath cells (SHIELDS 2008). The classification of sensilla is complex and also disunified, with some authors recognizing only a few and some many different classes. To avoid broaching the subject in finer detail, we suggest the classification of ALTNER & PRILLINGER (1980) as a solid foundation applicable across most insects. Compared with gland ducts, sensilla can be distinguished by the considerably larger diameter of their pore canal crossing the cuticle. Another characteristic is the presence of a central dendritic structure in their pore canal (black arrowheads in Fig. 11A - E), whereas the much smaller gland ducts (white arrowheads in Fig. 11B, E) have an empty lumen. In older literature until the mid '80s, when the existence of glands within the mandibles was not yet known, several gland structures have been regarded as sensilla in a dogmatic way (BIN & al. 1999). The other more recent interpretation of sensilla as glands (DO AMARAL & CAETANO 2006, MARTINS & SERRÃO 2011, MARTINS & al. 2015a) is to be considered as an unfortunate and misleading error.



**Fig. 10:** Transverse sections near mandible tip of workers of *Pseudomyrmex oculatus* (A), *Tetraponera allaborans* (B) and *Temnothorax ravouxi* (C) showing distal mandibular blade gland (DMBG). Note presence of numerous sensillar structures (S) at mesal side. Inset in (A): Mandibular surface of *Pseudomyrmex schuppi* worker with minute slits.



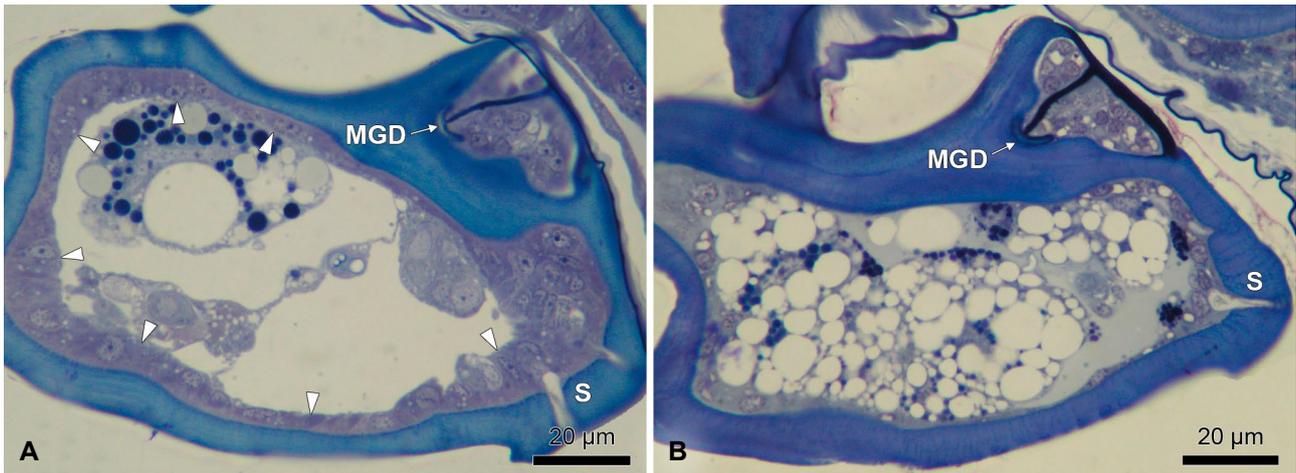
**Fig. 11:** (A) Longitudinal section through mandible of *Atta laevigata* major worker, showing several sensilla (S) with central dendritic structure (black arrowhead) in their large pore canal. The sensillar cells (SC) are very different from gland cells, although they were misinterpreted as such in the past (DO AMARAL & CAETANO 2006, MARTINS & SERRÃO 2011, MARTINS & al. 2015a). The asterisk indicates a sectioning artifact. ct: cuticle. (B) Sensillar structure in mandible of *Aneuretus simoni* minor worker. Note obvious difference in diameter of sensillar crossing through cuticle and ducts of class-3 gland cells (white arrowheads). (C) - (E) Transverse sections through mandibles showing sensilla that can be recognized by their broad crossing through cuticle and the occurrence of a dendritic nerve fibre (black arrowheads). Ducts of secretory gland cells (SC) have a much smaller diameter (white arrowheads). (C) *Liometopum microcephalum* worker with sensillar hair (sh). (D) *Solenopsis geminata* worker. (E) *Holcopena strigata* worker.

## Conclusion

Our survey of the intramandibular glands in ants reveals an overwhelming variety of exocrine structures. The discovery of the first glandular tissue in ant mandibles was made 30 years ago (SCHOETERS & BILLEN 1994) and at that time led to its straightforward but very general description “intramandibular gland”. Both technical improvements using hard embedding resins to allow sectioning hard insect cuticle (BILLEN & MORGAN 1998) and more thorough screening of numerous ant species have contributed to study the exocrine variety as outlined in this review. The discovery of ten more glands over the past three decades has necessitated the use of increasingly specific names

with accurate anatomical terminology (Tab. 1). Two of these glands (the ventrolateral intramandibular gland and the distal mandibular blade gland) are newly discovered and reported here for the first time. They add to the already phenomenal exocrine repertoire of ants, bringing the overall number to 115 currently described glands among the Formicidae.

In contrast to the mandibular gland, which consists of secretory cells that often open into a large reservoir sac, none of the observed intramandibular glands has a reservoir. The only possible exception could be the mandibular pit gland (BILLEN & AL-KHALIFA 2016), although the pit space and its wide contact with the exterior appear more



**Fig. 12:** Transverse sections through proximal mandible region of *Monomorium pharaonis* queens. MGD: mandibular gland duct, S: sensilla. Note thicker epithelium at eclosion (arrowheads). (A) 0 days old, (B) 2 weeks old.

like an evaporatorium and can hardly be regarded as a proper – that is, long-term – storage place for secretion. As discussed above (see “12. Contentious interpretations in literature”), the published reports on the presence of reservoir-containing glands in Attini was an erroneous interpretation of sensilla (DO AMARAL & CAETANO 2006, MARTINS & SERRÃO 2011, MARTINS & al. 2015a). Because of the absence of a reservoir, it is difficult to collect and chemically analyse the glandular secretion, hence it is challenging to unravel the function of the various intramandibular glands. Several histochemical studies of intramandibular glands report the presence of carbohydrates, proteins, and lipids leading the authors to the rather trivial conclusion that the gland cells display high secretory activity (MARTINS & SERRÃO 2011, MARTINS & al. 2015a, 2016). A role in chemical communication has been postulated (ROUX & al. 2010, MARTINS & al. 2015a, b, 2016), although convincing evidence in general is still lacking.

Despite the obvious diversity of intramandibular glands, the occurrence of spatially localized secretory tissues shows a narrow phylogenetic distribution as the majority of glands has been reported in a single species or genus (Tab. 1). The simultaneous occurrence of multiple intramandibular glands in the same species is generally restricted to two or three glands at most. The apparent similarity between some glands raises the question of their homology. For glands composed of class-1 cells, their location and peculiar association with the cuticle provide sufficient evidence for the proposal of convincing homology hypotheses. In particular, the ultrastructural appearance reveals further distinctive features. For example, some class-1 glands in *Strumigenys* (apicomasal intramandibular gland and ventral basimandibular gland) display the exclusive and threefold characteristics of a bowl shape, the presence of a cytoplasmic layer between the microvilli and the cuticle, and the presence of an obvious nerve fibre wedged in between the gland cells. This set of characters possibly reflects a special case of shared gene expression (WANG & al. 2023). It is likely that

the apicomasal and ventral basimandibular glands are serial homologies with distinct identity as *Strumigenys membranifera* clearly possesses both glands (note that *S. membranifera* is the only species in which the apicomasal intramandibular gland has been described).

It is important to clarify that the occurrence of a thickened epithelium does not always correspond with glandular function as freshly eclosed adult ants usually have such thick epithelium, reflecting its involvement in cuticle production (Fig. 12). For glands composed of class-3 cells, the variety of intramandibular glands is lower as only four are known to date. They can be relatively easily distinguished by their peculiar appearance and phylogenetic distribution (e.g., the basimandibular sieveplate gland is found in *Tatuidris*, the apicomasal gland only in queens of *Strumigenys mutica*, and the intramandibular silk gland only in *Rhopalomastix*). The dispersed intramandibular gland cells represent the most widespread as they have been found in workers, queens, and males of at least nine subfamilies (SCHOETERS & BILLEN 1994). Our review updates the occurrence of these dispersed intramandibular gland cells in 13 subfamilies. The only exception was *Tatuidris tatusia* (Agroecomyrmecinae); however, this species possesses a unique basimandibular sieveplate gland (BILLEN & DELSINNE 2014), which may represent a modification of the dispersed gland cells through the clustered occurrence and sieveplate opening of the ducts. All four described glands in *Strumigenys* can be considered as a differentiation of the ancestral subepidermal glands that also have a wide phylogenetic distribution (GOBIN & al. 2003).

Considering the first discovery of exocrine tissue inside ant mandibles dates back three decades (SCHOETERS & BILLEN 1994), the current description of ten additional glands clearly represents a major expansion of the intramandibular exocrine diversity of ants. The variety of 11 intramandibular glands as illustrated in this review, however, should not be seen as being the complete picture. In the future, more intramandibular glands will likely be discovered:

(1) Extending the number of examined taxa will give a better view of gland occurrence and diversity. Although the material studied in this review does cover a large phylogenetic range, it still is very fragmentary. To give just one example: The subfamily Myrmicinae comprises 147 genera and 7200 described species (BOLTON 2024), yet our study looked at a small fraction of this represented by 9 genera and 18 species (Tab. 2). A thorough examination of a particular taxon, however, illustrates the potential exocrine wealth as was done for *Strumigenys*, with three of the 11 intramandibular glands here reported as exclusive for this genus (BILLEN & ESPADALER 2002, WANG & al. 2021c). Systematic sectioning of anterior head parts and active searching for glands of particular species can be an almost endless approach, but can be very rewarding.

(2) It has to be stressed that the present review is largely based on workers (89% of the 266 examined specimens in this review are workers, 9% are queens, and hardly 2% are males). Although this proportion may reflect the general situation of sex and caste studies in ant literature, expanding the number of queens and especially males should provide a better insight in gland diversity.

(3) More scanning microscopy in search for external mandibular characteristics such as smooth patches (with minute slits that can indicate presence of class-1 glands: BOLTON 1999) or pores with a diameter ranging between 0.5 and 1 µm (for class-3 glands) can lead to new discoveries of intramandibular glands.

(4) The function of only a few intramandibular glands is known. Observation of conspicuous mandible-associated behaviours may possibly lead to glandular functions that can then be histologically verified. It may be interesting in this regard to study species living in different ecological conditions and habitats as these may influence mandibular function. The discovery and structural description of intramandibular glands in future work will hopefully be coupled with analysis of the chemical composition of their secretions and lead to a better understanding of their function.

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## Declaration on use of generative artificial intelligence tools

The authors declare that they did not utilize generative artificial intelligence tools in any part of the composition of this manuscript.

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