Morphology and closing mechanism of the mandibular gland orifice in ants (Hymenoptera: Formicidae)

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Abstract
The mandibular gland of ants releases chemical compounds with functions ranging from nestmate alarm and recognition to antimicrobial defense. While the morphology of this ethologically important gland is well investigated in several species, the mechanism of secretion release in ants was not explicitly addressed so far. To clarify this question, we examined the anatomy of the gland orifice in ant species from 14 different subfamilies employing different techniques. The orifice close to the mandibular base is located on an area called mandalus. Our investigations revealed variation in mandalar shape, with clear trends in different subfamilies. By contrast, the internal organization is remarkably congruent across all investigated species. The thin external mandalar cuticle is always connected to the mandibular gland duct by a cuticular lamella, visible as a characteristic anchor-shaped structure in cross section. The slit-like gland orifice at the distal end of the mandalus is usually crescent-shaped. In some ant species with specialized mandibles such as trap-jaws, the organization of the orifice area is adapted to the mandibular shape, but always retains the general internal organization. No muscles were found in association with the orifice, nor with any other part of the mandibular gland. However, the base of the mandalus is connected to the prepharyngeal sucking pump by a cuticular ligament. Additionally, it is continuous with the conjunctiva connecting the mandible to the head capsule. We propose that retraction of the sucking pump by the muscle M. tentoriobuccalis, potentially in concert with opening of the mandible, stretches out the ligament and thus pulls on the mandalus and mandalar lamella to open the gland orifice and allow for secretion release. This hypothesis is congruent with findings on other aculeate Hymenoptera and expands our knowledge on the function of an important gland of ants.

KEYWORDS
alarm-defense system, functional morphology, glandular orifice, mandalus, mandibles, pheromones
1 | INTRODUCTION

Ants have an elaborate array of exocrine glands, which are important for many aspects of their biology and social life (e.g., Billen & Morgan, 1998; Hölldobler & Wilson, 1990). One of them, associated with the mandibles, also occurs in other groups of insects, as for instance in many other Hymenoptera (e.g., Singh & Singh, 1982) and polyphagan beetles (Anton & Beutel, 2004). This paired gland produces a wide range of different secretions in ants (e.g., Blum et al., 1968; Brückner et al., 2018; Hogan et al., 2017; Morgan et al., 1999; Sainz-Borgo et al., 2013), serving several confirmed functions in different ant species, such as alarm of nestmates (Maschwitz, 1964: several species of Myrmicinae, Formicidae and Dolichoderinae, honeybee; Brough, 1978: Calomyrmex Emery, 1895; Billen & Morgan, 1998: several myrmicine species and other Aculeata; Billen et al., 2013: Leptanilla sp.), nestmate recognition (Sainz-Borgo et al., 2013: Acromyrmex landolti [Forel, 1885]), attracting mating partners (Lloyd et al., 1975: male Camponotus clarithorax Creighton, 1950; Greenberg et al., 2004: queen Polyrugus breviceps Emery, 1893), host manipulation by social parasites (Viscichio et al., 2001: Polyrugus rufescens [Latreille, 1798]), antimicrobial defense (Knapp et al., 1994: Acromyrmex Mayr, 1865, Atta Fabricius, 1804; Brough, 1983: Calomyrmex Emery, 1895; de Lima Mendonça et al., 2009: Atta) and even as a direct weapon in the “exploding ants” (Davidson et al., 2012: Colobopsis cylindricus complex).

The mandibles of ants are incredibly diverse and come in many different shapes (for an overview see, e.g., Gotwald, 1969). An important property of these mouthparts is that they can be opened independently of the maxillolabial complex (Keller, 2011). Opening movements of the mandible can be observed not only when they are used to grab objects, brood or prey, but also when the ant is confronted with opponents or when it tries to alert nestmates (e.g., Howard et al., 1982; Maschwitz, 1964).

The articulation of the mandible consists of two joints as it is generally the case in dicondylic insects with well-developed mouthparts. This confines the movement of the mandible to one plane. However, Richter et al. (2019) documented that the secondary (dorsal) mandibular joint is distinctly modified as an elongated sliding surface, indicating that the degrees of freedom for mandibular movement could be increased compared to other dicondylic insects. Indeed, analyses of Zhang et al. (2020) suggest that the ant mandible at least in the genus Harpegnathos Jerdon, 1851 has a biaxial rotation in two planes. Two muscles, a large adductor and a smaller abductor are responsible for mandibular movements (Matsuda, 1965; Paul, 2001; Richter et al., 2019, 2020), as it is also the case in other dicondylic insects.

The paired mandibular glands are formed by a cluster of glandular cells that release their secretion into a reservoir, and a main duct that connects the reservoir with the exterior (e.g., Billen & Al-Khalifa, 2018; Billen & Schoeters, 1994). This duct opens on the mandalus, a membranous field on the (proximal) dorsal mandibular base. This specific region has been described in detail in relatively few studies (e.g., Gotwald, 1969; Grasso et al., 2004; Richter et al., 2019, 2020) as it is usually hidden below the clypeus. This region is of particular functional interest to understand how the secretions of the mandibular gland are released. How exactly this is achieved has not been clarified in ants thus far, even though several hypotheses have been proposed to explain this problem in different groups of Hymenoptera; Simpson (1960) and Nedel (1960) suggested that secretion release in several bee species can be achieved through an effect caused by hypopharyngeal depression on a thread-like connection with the gland opening. Kratky (1931) proposed mandibular abduction as potential mechanism in the honeybee. The latter hypothesis was also adopted by Grasso et al. (2004) for ants, who proposed that increased hemolymph pressure during mandibular opening could lead to release of gland secretion. Boonen et al. (2013) proposed that a cuticular lamella connecting the gland duct to the mandalus could play a role in the release mechanism.

This study developed after initial detailed histological investigations on Atta sexdens and Monomorium pharaonis. In these two species, we first noticed the curious internal shape of the mandalar region and the complete absence of muscles connected to the mandibular gland and its orifice. To further analyze and understand the shape of the mandalar region, Atta was also examined with scanning electron microscopy and we compared our initial observations with a large species of Dinoponera sp.

To better understand these initial observations, we compared the anatomy of the mandibular base containing the gland orifice and the mandalus in members of most ant subfamilies using scanning electron microscopy, histological sections and also micro computed-tomography (µCT)-scan based 3D-reconstruction of one representative species. Based on our morphological data we discuss the mechanism for release of glandular secretion.

2 | MATERIALS AND METHODS

2.1 | Material examined

Information on the species investigated, localities, collectors and collection codes and storage location as well as investigation techniques can be found in Table S1. No special permits were necessary at the time of collecting the specimens, except for the recently collected Leptomyrmex unicolor from Australia, for which permission was granted under the number PTU19-002400 by the Department of Environment and Science, Queensland, Australia. Overall, representatives of 14 subfamilies were investigated, all of them with histological sections and eight also with SEM. Our taxonomy follows Bolton (2021), which means subfamilies are supported as monophyletic by the most recent molecular phylogenetic analyses (e.g., Borowiec et al., 2019; Moreau & Bell, 2013).

2.2 | Histology and scanning microscopy

Heads (cut open around the vertexal region for better fixation) were fixed for 2–20 h in 2% glutaraldehyde (4°C, pH 7.3 and buffered with 0.05 mol l⁻¹ sodium cacodylate). Postfixation in a buffered osmium
tetroxide solution (1 h) was followed by dehydration in an acetone series and embedding in Araldite. Semi-thin sections (1 μm thickness) for light microscopy were made using a Leica EM UC6 ultramicrotome and stained with methylene blue and thionin.

Samples for scanning microscopy (SEM) were dehydrated in an ethanol series after postfixation and were critical point dried. They were coated with gold and viewed in a Philips SEM 515 microscope. Part of the samples for SEM also consists of material collected and preserved in 70% ethanol. Scanning electron micrograph negatives were digitized using a Maginon F5600 film scanner (Kaiserslautern, Germany).

### 2.3 | μCT and 3D-reconstruction

One specimen of *Leptomyrmex unicolor* (collected in QLD Australia, rainforest, –16.102306, 145.448079 by P.T. Rühr, 09.11.2019) was identified using the key on antwiki.org based on Smith and Shattuck (2009) and Lucky and Ward (2010). It was stored in 70% ethanol. For μCT-scanning it was first transferred into 100% ethanol through a rising ethanol series (80%, 90%, 96%, 100%) before being stained in a 1% iodine solution in pure ethanol for 4 days. After staining, it was put into 100% acetone and subsequently critical point dried in liquid CO₂ with an Emitech K 850 Critical Point Dryer (Sample preparation Division, Quorum Technologies Ltd., Ashford, England). The μCT scan was performed using a Skyscan 2211 (Bruker, Belgium) at the Max Planck Institut for the Science of Human History Jena, equipped with a high resolution (4000 × 2600 pixel) X-ray sensitive CCD camera. A beam strength of 50 kV and 290 μA was employed in the nanofocus mode with an exposure time of 4 s. An image pixel size of 0.7 μm was chosen in a 360° scan with 0.18° rotation steps. Two images of every position were averaged. A focus current test was performed and the focus current setting resulting in the sharpest image was chosen. Tomographic reconstruction was done using NRecon (Version: 1.7.3.1) and exported as a 16-bit TIFF image series. After binning and transforming to 8-bit images, the isotropic voxel size was 1.4 μm.

The head capsule, mandible, cephalic digestive tract, mandibular gland duct, as well as the mandibular and its associated structures were segmented using Amira 6.0 (Visage Imaging GmbH, Berlin, Germany). Head capsule and mandible were pre-segmented on every 20th slice and segmentation was finished semiautomatically using biomedisa (Lösel et al., 2020). The resulting materials were exported with the plugin script “multiExport” (Engelkes et al., 2018) in Amira 6.1 as Tiff image stacks. VG Studio Max 3.4 (Volume Graphics GmbH, Heidelberg, Germany) was used for volume rendering based on the image stacks.

### 2.4 | Image processing

Figure plates of SEM micrographs, histological sections and volume renderings were assembled in Adobe Photoshop® CS6 (Adobe Systems Incorporated, San Jose, United States). Labels were added in Adobe Illustrator® CS6 (Adobe Systems Incorporated). Original hand drawn images were scanned, reproduced and modified digitally using Adobe Illustrator® CS6 (Adobe Systems Incorporated).

### 2.5 | Terminology

Terminology generally follows Richter et al. (2020). We term the thin cuticular regions connecting the mouthparts with the head capsule “conjunctiva” in accordance with the Hymenoptera Anatomy Ontology (Yoder et al., 2010).

### 3 | RESULTS

#### 3.1 | Anatomy of the mandibular gland opening and mandalus

In all species studied, the orifice of the mandibular gland is found in the region of the mandalus, which is close to the proximal upper margin of the mandible (Figure 1a,c). The cuticle of the mandalus is much thinner and more flexible than that of surrounding mandibular areas. The slit of the gland orifice marks its distal border. The mandalus is a part of the flexible conjunctiva connecting the mandible to the head capsule, that forms a flap covering a depression in the sclerotized mandibular cuticle (Figures 2c and 3a,b). The mandalus is located directly mesad the mandibular acetabulum in most species, which represents the secondary (dorsal) articulation point of the mandible (dma, Figures 1a,b and 2b). The part of the mandible mesad of the mandalus, which is sometimes thickened (e.g., Acromyrmex crossospinus, Figure 4), is often referred to as the canthellus (ca, Figure 1c). In many species, a groove or depression can be observed directly distad of the mandalar region. In species of Myrmicinae, this is usually a triangular depression with several small sensilla, called the trulleum (Figures 1c and 4).

The mandalus is usually surrounded, at least partly, by an externally visible distinct slit. While the distal region of this fissure represents the mandibular gland orifice, the more proximal part is usually the point of fusion of the mandalus with the mandibular groove it covers (mas, Figure 3b). This slit sometimes surrounds the mandalus almost completely, for example, in *Formica rufa* and *Aphaoenogaster senilis* (Figure 4). It is important to note that the proximal slit has no connection to the gland duct. The distal slit forming the gland orifice is usually crescent-shaped and can vary in width. It is not always clearly visible externally on SEM images, indicating that it is either tightly closed or plugged with secretion. In some species, the distal orifice of the slit appears continuous with a lateral slit reaching all the way to the base of the mandalus. However, in these cases there is always an internal point of transition where the slit represents the fusion of the mandalus to the mandible (mas, Figure 3b) instead of the opening of the mandibular gland duct (mdgo, Figure 3a). At its mesal base, the mandalar cuticle is connected to a ligament. This ligament is relatively thick close to the mandalus; it curves around the conjunctiva connecting mandible and head capsule (mali, Figures 2a,b and 3c,d) and from this point connects straight to the sitophore plate, which is
the ventral prepharyngeal wall (sp, Figure 3d). The exact position at which the ligament connects to the prepharynx varies slightly across species. In most investigated species it is in a similar position to Leptomyrmex (Figure 2a), close to the buccal tube, around the level of insertion of the clypeopalatalis b muscle (Oc11b, Figure 3d), sometimes slightly further posterior around the level of the clypeobuccalis muscle (Obu1).

Histological sections, the µCT-scan and experimental cleavage of the mandibular cuticle in large ants of the genera Dinoponera and Atta allow to relate the external morphology of the mandalar region to the internal mandibular gland duct. A longitudinal cleavage reveals that the duct consists of two parts; near the orifice on the mandalus it is slit-like, crescent-shaped and sinks into the depression of the mandibular cuticle (Figures 1d,e, 2c and 3a,b), whereas closer to the mandibular gland reservoir it is expanded and has a wrinkled appearance closely resembling that of the mandibular gland reservoir itself (Figures 1e and 3d). The distinct transition between the flat lining of the narrow distal part and the wrinkled cuticular wall lining the duct of the mandibular gland closer to the reservoir is visible on SEMs of the cleavage preparations (Figure 1d,e). The distal region of the duct is connected to a cuticular lamella fused with the mandalus and more posteriorly the mandalar ligament above it (la, Figures 1d,e, 2 and 3a-c). This gives the distal part of the gland duct a characteristic anchor-shaped appearance in cross section (Figures 2c, 3b and 5).
the mandibular gland duct orifice and the general shape of the mandalar region is of variable shape in different species, but the internal anchor shape is consistent across all samples we investigated. This does not only include different species, but also different castes (workers, queens and males). The preparations, μCT data and histological sections also revealed the complete absence of muscular elements associated with the mandalus, the duct, or any other part of the gland.

3.2 | Comparison of the mandalar region in the different subfamilies

The internal organization of the mandibular gland orifice is consistent across different ant species (Figure 5). However, we were able to observe some differences in the shape of the cuticular flap covering the gland orifice, the mandalus. As the mandalar shape is often similar within the ant subfamilies, the shape differences across the investigated formicid subgroups will be detailed here according to subfamily. A short description of mandalus shape of all species investigated by SEM or μCT is provided in Table S2. As the investigated trap-jaw species of different subfamilies are characterized by unique mandible shapes, their mandalus is described in a separate section (Trap-jaw ants). Our findings are summarized in Figure 4 (some additional taxa can be seen on Figure S1).

The mandalus is a well-defined plate-like structure of varying shape in the investigated members of the Formicinae; it is roughly rectangular in most Camponotus, Cataglyphis and Lasius, sometimes narrowing proximally; its orientation is slightly oblique with the tip
facing laterad; it is strongly narrowed proximally in the investigated Formica species, resulting in a more spatulate shape. The mandalus is unusually large in some species of Lasius and Lepisiota, covering roughly half of the mandibular base (Fig. S1). The distal margin is at least slightly convex in all examined species, and the slit of the gland orifice is usually distinctly visible.

The slit is long in some species (see Lasius flavus), extending onto the mesal margin of the mandalus. An additional slit is sometimes present on the proximal region of the mandalus (e.g., L. flavus worker).

The mandalus is usually short in Myrmicinae, rectangular, spatulate or almost circular. In most species, it is directly bordered by the trulleum distally, and oriented straight along the mandibular base or slightly oblique. An exception is Strumigenys membranifera, where the trulleum is missing and an elongated spatulate mandalus is placed more on the mesal than on the dorsal side of the mandible. The slit of the distal gland orifice is always at least slightly curved and often connected with a mesal slit along the mandalar margin (e.g., Acromyrmex crassispinus).

The investigated species of Ectatomminae have a strongly curved, spatulate mandalus with a distinct slit along the entire mesal margin.

Dolichoderinae have a rather broad, roughly paddle-shaped mandalus which is distinctly curved laterad. The slit is broad and surrounds the entire mandalus, but only the distomedial part represents the actual gland orifice.

Pseudomyrmecinae have a short J-shaped mandalar region with a similar shape to dolichoderines but smaller and less curved.

The mandalus of representatives of the Myrmicinae is elongate spatulate and oriented almost completely straight along the mandibular base. It is distally bordered by a distinct groove somewhat resembling the myrmicine trulleum, but less clearly demarcated. The
The mandalus of *Nothomyrmecia macrops* is curved instead of straight, and the broad distal part is enlarged, covering the dorsal mandibular base almost completely. The distinct groove observed in *Myrmecia* is absent. The mandalus is narrow in species of *Dorylinae*. It is usually represented by a long, thin proximal slit in the mandibular cuticle, which slightly widens into a spatulate typical mandalar plate of varying length. The entire mandalus is always distinctly curved.

A remarkable diversity of mandalus shape is found in *Ponerinae*. It can be similar to the mandalus of dorylines, but the plate-like distal part is often larger. The slit in the mandibular cuticle can be shorter and the curve of the mandalus more sharply angled. In some cases the

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**FIGURE 4** Scanning electron micrographs of the dorsal mandibular bases of a selection of ant species giving an overview over the different shapes of the mandalus (marked in yellow). Formicinae: *Lasius flavus* worker (W), queen (Q) and male (M), *Formica rufa*, *Cataglyphis mauritanicus*; Myrmicinae: *Aphaenogaster senilis*, *Acromyrmex crassispinus*, *Strumigenys membranifera*; Ectatomminae: *Ectatomma opaciventris*; Dolichoderinae: *Technomyrmex* sp.; Myrmeciinae: *Nothomyrmecia macrops*, *Myrmecia pilosula*; Dorylinae: *Labidus praedator*, *Aenictus asantei*; Ponerinae: *Diacamma ceylonense*, *Megaponera foetens*, *Odontomachus simillimus*; Leptanillinae: *Leptanilla japonica*. 

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FIGURE 5  Histological sections through the mandibular bases at the level of the mandalus of a selection of ant species giving an overview over the uniformly anchor-shaped internal morphology. All mandibles of the right side of the head. Formicinae: Formica rufa, Oecophylla longinoda; Myrmicinae: Solenopsis geminata, Aphaenogaster senilis, Strumigenys mutica; Heteroponerinae: Heteroponera dolo; Ectatomminae: Gnamptogenys strigata; Aneuretinae: Aneuretus simoni; Dolichoderinae: Tapinoma nigerrimum; Myrmeciinae: Nothomyrmecia macrops; Pseudomyrmecinae: Pseudomyrmex ferrugineus; Dorylinae: Labidus coecus, Dorylus orientalis; Amblyoponinae: Stigmatomma roahady; Proceratiinae: Proceratium japonicum; Agroecomyrmecinae: Tatuidris tatusia; Paraponerinae: Paraponera clavata; Ponerinae: Myopias conicara, Cryptopone taivanae; Leptanillinae: Leptanilla clypeata

Formica rufa  Oecophylla longinoda  Solenopsis geminata  Aphaenogaster senilis

Strumigenys mutica  Heteroponera dolo  Gnamptogenys strigata  Aneuretus simoni

Tapinoma nigerrimum  Nothomyrmecia macrops  Pseudomyrmex ferrugineus  Labidus coecus

Dorylus orientalis  Stigmatomma roahady  Proceratium japonicum  Tatuidris tatusia

Paraponera clavata  Myopias conicara  Cryptopone taivanae  Leptanilla clypeata
mandalus is almost straight, with an oblique orientation. spatulate shapes resembling the condition in Formica also occur.

The shape of the large mandalus of species of the genus Leptanilla of Leptanillinae is distinctly modified. proximally and distally, it consists of thin cuticle as in other ants, but centrally it is “interrupted” by thick cuticle that is only slightly thinner than that of the surrounding mandible (Figure 5). This region remains connected to the lamella holding the gland duct. It is unclear whether it is part of the actual mandalus, but for simplicity we marked it as such on Figure 4. The slit-like orifice of the mandibular gland is broad as the mandalus. Like the mandalus, the mandibular gland duct is also large, with a particularly long lamella, filling out a significant part of the mandibular lumen (Figure 5).

3.3 | Trap-jaw ants

trap-jaw ants have some of the most peculiar mandible shapes observed in the family and this also leads to interesting modifications of the mandibular gland orifice in some of these species. Most prominently this can be seen in the ponerine genera Odontomachus and Anochetus. Due to the distinct modification of the mandibular base, linked with the formation of a latch mechanism, the position of the mandalus is shifted to a dorsomesal position on the mandibular base. the mandalus consists of a long, thin, indistinct slit on the mesal side of the mandible ending in a small patch of thin mandalar cuticle with the gland orifice (Figure 4, Odontomachus simillimus and Figure 6, Anochetus sp. and O. assiniensis). the internal organization is generally the same as in all other investigated ants, with a lamella connecting the gland duct with the mandalus, but due to the mandalar orientation there is a distinct curve in the lamella connecting to the gland duct. Slightly less extreme modifications occur in the myrmicine trap-jaw species. in Daceton armigerum, the mandalus is developed dorsally on the mandible similar to other myrmicines (Figure 4), although the myrmicine trap-jaw species are notably missing the trulleum. Another special case is the formicine Myrmoteras iriodum (Figure 4). In this species, the mandibular gland duct runs mesodorsally on the mandible proximally. In this area, the whole mesal wall of the mandible is made up of very thin cuticle (see also Larabee et al., 2017). More distally, the main area of the mandalus is on the dorsal side of the mandible.

4 | DISCUSSION

4.1 | Evolution of the mandibular gland duct orifice and related mandibular regions in Formicidae

we observed considerable variation of mandibular gland openings in species of different ant lineages, but the fundamental morphological configuration is conserved. our study reveals a tendency toward a narrower and more elongated mandalus in species with longer mandibles and mostly predaceous habits (e.g., army ant soldiers, Myrmeciinae, Ponerinae, Strumigenys), and relatively wide, plug-like structures in species with shorter mandibles and less specialized habits. however, exceptions occur, such as the predatory Notomymecia macrops with

![Image of histological sections through mandibular bases at the level of the mandalus of several trap-jaw ant species.](image-url)
 elongated mandibles and a large, plug-like mandalus. No correlations between the shape of the mandible and gland orifice with the function of the gland secretion were obvious based on our qualitative results. However, the exact function of the secretion has been revealed for relatively few taxa so far, mostly focused on Myrmicinae and Formicinae. The most common function also found in other Aculeata seems to be alarm of nestmates (e.g., Maschwitz, 1964). It would be interesting, for example, to study the function of the gland secretion of different trap-jaw ant species, some of which we show to have a rather unusual configuration of the gland orifice relative to other ant species. Trap-jaw mandibles can fulfill several different roles such as devices for prey capture and escape jumps (Larabee & Suarez, 2014).

Overall, the differences in shape are consistent on the generic level and rough trends also correspond with the subfamily level. Most common across the species we investigated is a spatulate, usually at least slightly curved or angled shape. This occurred in species of Dorylinae, Ectatomminae, Formicinae, Myrmeciinae, Myrmicinae and Ponerinae. While in Ponerinae and especially Dorylinae the mandalar area is rather narrow and curved, it is more rounded and plug-like in Dolichoderinae, Pseudomyrmecinae and Formicinae, although Formicinae also show narrow spatulate shapes relatively similar to ponerines (e.g., in Formica) and more rectangular rather than rounded mandalar plates (Cataglyphis). The Myrmicinae that we investigated (except for Strumigenys and Trachymyrmex) are characterized by a short, roughly rectangular mandalus. Myrmeciinae, except for Nothomyrmecia (with a distinct curve and a large, round plate), show a distinctly straight spatulate shape. Comparison with the literature reveals that even more unusual configurations of the mandibular gland opening area exist. The mandibular groove containing the distal gland duct of Protanilla lini is expanded as a long tunnel through the mandibular base opening on the lateral side, a unique position for the gland opening compared to all species we studied (Richter et al., 2021). Nevertheless, the internal configuration is the same as described here for other ants, with a lamella connecting the mandibular gland duct to the cuticle of the tunnel resulting in the typical anchor shape in cross section. This raises the question if thick, sclerotized cuticle can be considered as part of the mandalus if it is connected to the mandalar lamella internally. A similar case was also observed in the closely related Leptanilla, in which the area of the presumable mandalus (see Figure 4) partly consists of thick, likely inflexible cuticle. Studies on the development of the different involved structures could possibly help to reveal their exact identity and origin, even when they are strongly modified such as in these examples. As another interesting difference to the species investigated by us, the ligament connecting to the prepharynx inserts much further posterior around the level of frontobuccalis muscle (0bu2) on the oral arms in Protanilla lini, indicating that there may be more variation of this feature hidden in ant diversity. It is conceivable that the shape of the mandalus is determined by the general mandibular shape. An investigation with a larger taxon sampling could reveal a strict correlation, but also additional or alternative phylogenetic or ecological constraints.

While a flat mandibular groove close to the mandalus is present in some species of Formicinae, Dolichoderinae and Myrmeciinae, a “truleum” occurs only in species of Myrmicinae, contrary to descriptions of previous authors (e.g., Gotwald, 1969). Richter et al. (2019) suggested that the truleum might be a simple consequence of the canthellus meeting with the basal mandibular margin, thus representing a “spandrel” without a function of its own (Gould & Lewontin, 1979). Some authors (Buren et al., 1970; Snodgrass, 1956) suggested that a mandibular groove could serve as transport route for the gland secretion toward the mandibular tip, but arguments against this hypothesis have also been made for Vespidae (Hermann et al., 1971). We cannot completely exclude that the grooves occurring in different ants, including the truleum of Myrmicinae, are functionally linked with the release of mandibular gland secretions. However, the complete absence of such grooves in several investigated subfamilies makes it unlikely that it is essential for the proper function of the gland.

4.2 | Mechanism of secretion release of the mandibular gland

The complete absence of musculature directly connected to the mandibular gland or its opening region raises the question how the gland secretion can be released without direct mandibular control. A thin lamella connects the gland duct and the mandalus. The mandalus itself is made up of thin, flexible cuticle. It is close to the oral foramen and continuous with the conjunctiva which connects the mandibular base with the head capsule, and also continuous with a ligament connected to the prepharynx. Our hypothesis for the opening mechanism of the orifice is illustrated in Figure 7. The prepharynx, more precisely its ventral (hypopharyngeal) wall can be pulled backwards by the contraction of the ventral buccal dilator (M. tentoriobuccalis, 0bu5). Retraction of the ventral prepharyngeal wall leads to a backward movement of the ligament connected to the mandalus. The lamella connecting the mandalus and its ligament with the distal mandibular gland duct transmits forces on the latter. We thus hypothesize that by retracting the prepharynx, ants can indirectly pull open the slit-like orifice of the mandibular gland duct at the same time expand the duct, allowing release of the mandibular gland secretions. It is conceivable that opening of the mandible also results in some pull on the mandalus, transmitted by the conjunctiva connecting the mandible with the head capsule, and also by straightening the curved ligament to a certain degree. However, it is unlikely that opening the mandible alone is sufficient for opening the gland orifice. It is possible that opening of the orifice requires the interplay of prepharyngeal retraction and opening of the mandible or is at least facilitated by the latter. In some ant species, such as the trap-jaw ants Odontomachus and Anochetus, as well as the leptanilline Protanilla lini discussed above, the morphology of the mandalus is strongly modified. However, the consistent presence of the lamella connecting mandalus and gland duct even in these species likely ensures a similar interaction of the gland orifice with the ligament pulling on the mandalus, allowing the proposed opening mechanism to work in these genera as well.

Several hypotheses on the release of mandibular gland secretions have been suggested, notably a mechanism related to opening
movements of the mandible (Billen & Schoeters, 1994; Grasso et al., 2004; Kratky, 1931). Hernández and Caetano (1995), for instance, suggested this in a study on Atta. In particular, they argued that the gland orifice and tralleum are hidden below the clypeus when the mandibles are in a flexed position, thus preventing secretion release. Opening of the mandible would expose the gland orifice and tralleum, thus enabling release of gland substances. Boonen et al. (2013) observed the presence of the cuticular lamella connecting the flexible cuticle of the mandalus with the mandibular gland duct. The authors discussed a possible link with the opening mechanism, assuming that extension of the mandible would stretch the conjunctiva connecting the mandibular base (including the mandalus) with the head capsule. This movement would be transferred to the mandalus and through the lamella connected to the mandibular gland duct also indirectly to the duct and its orifice. Such a mechanism seems to agree with the ideas of Kratky (1931), Billen and Schoeters (1994), Grasso et al. (2004) and Boonen et al. (2013), although none of these authors explicitly described the hypothesized mechanism in detail.

Simpson (1960) proposed the involvement of hypopharyngeal depression as a hypothesis for the release mechanism in Apis mellifera. He found a “fibrous band” connecting the gland orifice with the “hypopharyngeal plate” and observed that depression of the hypopharynx with a probe led to release of gland secretion. Nedel (1960) described essentially the same mechanism for the honeybee queen as well as stingless bees of the genera Trigona and Scaptotrigona. For the stingless bees, he explicitly described an elastic chitinous plate closing the orifice of the glandular duct and inhibiting the release of secretion. After being pulled open, this plate returns to its initial position, facilitated by its elastic properties. Nedel (1960) observed two slender apodemes involved in opening the orifice of the honeybee queen, but otherwise his observations are congruent with those of Simpson (1960). The descriptions of both authors are consistent with our hypothesis of the opening mechanism in ants. Even though the term “mandalus” is restricted to the myrmecological literature (see, e.g., Hymenoptera Anatomy Ontology), Nedel’s descriptions and interpretations confirm that a similar configuration also occurs in other aculeates. The description of a “fibrous band” connected to the hypopharynx (Nedel, 1960; Simpson, 1960) agrees with our observation of a ligament connecting the gland orifice to the ventral prepharyngeal wall, which is homologous with the proximal hypopharynx (e.g., Beutel et al., 2014; Richter et al., 2019). We thus suggest that the release mechanism in ants and investigated bee species functions in a similar way and is likely homologous. Consequently, the opening of the mandibular gland is facilitated indirectly by the action of M. tentoriobuccalis (0bu5), the only muscle that is positioned to pull back the relevant part of the alimentary canal (e.g., Paul et al., 2002). The structural similarity of the mechanism in bees and ants could be interpreted as additional morphological evidence for the sister group relationship of Formicidae and Apoidea (e.g., Peters et al., 2017). However, as this specific character system has not been investigated in other Aculeata so far, additional comparisons will be required. Such comparisons could also be interesting in the context of understanding the origin of the mandalar ligament. It might have originated as an outgrowth or evagination of the cuticle of the
hypopharynx, mandible, or both. Developmental analyses could also help to clarify this question.

While Nedel (1960) and Simpson (1960) hypothesize that the mandibular gland reservoir is under constant pressure and will thus immediately release gland secretions upon opening, an alternative view would be that opening of the gland orifice and expansion of the gland duct through the lamella connected to the mandalus create negative pressure leading to secretion release. Interestingly, the ligament has never been explicitly mentioned or described in studies on ants, a possible reason for the appeal of the alternative hypothesis related to mandibular opening. As mentioned above, we cannot completely exclude that extension of the mandibles is involved to a certain degree. As shown in our reconstruction of Leptomyrmex (Figure 2), the position of the ligament requires a strong retraction of the ventral prepharyngeal wall to induce a pull on the mandalus. However, with the mandible in an opened position, the ligament would be partly straightened already, indicating that both mechanisms could be most efficient when combined. Automatic opening with every extension of the mandibles would obviously result in wasteful release of the gland secretions. A dual mechanism, however, would have the advantage of ensuring a high degree of control of the release, which is likely beneficial in the context of energetically costly metabolites such as gland products. Ants often open their mandibles widely in alarm or stress situations in which the mandibular gland would presumably be in use (e.g., Howard et al., 1982: Wasmannia auropunctata), which would fit with this interpretation.

5 | CONCLUSION

The mandibular gland orifice is located on the mandalus. This area of thin cuticle located at the base of the mandible is of highly variable shape across different ant subfamilies and in species with differently shaped mandibles. However, the internal organization of this region is highly conserved across all ants investigated. The mandalus is connected to the duct of the mandibular gland through a cuticular lamella, giving a characteristic anchor shape in cross section. Additionally, the mandalus is connected to a cuticular ligament attached to the lower wall of the prepharynx (the hypopharynx). We propose that by retracting the prepharynx (via M. tentoriobuccalis Obu5) the ligament pulls on the mandalus and through the cuticular lamella also on the mandibular gland duct. This expands the duct and opens the gland orifice at the tip of the mandalus to allow for secretion release. A possible additional factor is mandibular opening. Through pulling on the mandalus by the conjunctiva connecting the mandibular base to the head capsule and straightening of the ligament this could also contribute to the opening of the mandibular gland, giving a double control for the release of metabolically expensive gland secretions. Our proposed ligament facilitated opening mechanism is congruent with older findings in different bee species but explicitly investigated and described in ants for the first time here.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Adrian Richter: Data curation; investigation; visualization; writing-original draft (second version); writing-review & editing. Eric Schoeters: Conceptualization; investigation; writing-original draft; -writing-review & editing. Johan Billen: Conceptualization; data curation; investigation; resources; writing-review & editing.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The μCT scan used in this study is openly available at the online repository Zenodo under the DOI 10.5281/zenodo.4623822. Information on storage and accessibility of the histological sections and samples used for SEM can be found in Table S1, unless otherwise noted material is stored at the Zoological Institute of the KU Leuven, Belgium. Access to material and original image data is available upon request to the corresponding author or J. B.

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**SUPPORTING INFORMATION**

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