

The ultrastructure of the intramandibular gland in soldiers of the termite *Machadotermes rigidus* (Blattodea: Termitidae: Apicotermitinae)

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ARTICLE INFO

Article history:

Received 4 September 2021

Accepted 10 December 2021

Available online xxx

Keywords:

Termites

Isoptera

Exocrine organ

Class III secretory cell

Mandibles

Soldier caste

ABSTRACT

Machadotermes is one of the basal Apicotermitinae genera, living in tropical West Africa. Old observations suggested the presence of a new gland, the intramandibular gland, in *Machadotermes* soldiers. Here, by combining micro-computed tomography, optical and electron microscopy, we showed that the gland exists in *Machadotermes* soldiers only as an active exocrine organ, consisting of numerous class III cells (bicellular units made of secretory and canal cells), within which the secretion is produced in rough endoplasmic reticulum, and modified and stored in Golgi apparatus. The final secretion is released out from the body through epicuticular canals running through the mandible cuticle to the exterior. We also studied three other Apicotermitinae, *Indotermes*, *Duplidentitermes*, and *Jugositermes*, in which this gland is absent. We speculate that the secretion of this gland may be used as a general protectant or antimicrobial agent. In addition, we observed that the frontal gland, a specific defensive organ in termites, is absent in *Machadotermes* soldiers while it is tiny in *Indotermes* soldiers and small in *Duplidentitermes* and *Jugositermes* soldiers. At last, we could also observe in all these species the labral, mandibular and labial glands, other exocrine glands present in all termite species studied so far.

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1. Introduction

Termites are eusocial cockroaches (Lo et al., 2000; Inward et al., 2007; Bourguignon et al., 2015), and one of the most successful organisms in terms of overall abundance (Bar-On et al., 2018). Termites are decomposers feeding on a broad range of vegetal matter from living tissues (fungi, lichens) through moderately decayed materials (wood, dry grass, leaf-litter, etc.) to the mineralised organic compounds in bare soil (Donovan et al., 2001; Eggleton and Tayasu, 2001; Bourguignon et al., 2011). The “Prototermite” surely lived in dead wood (Chouvenc et al., 2021), a stable food source for increasing numbers of family members. Once this

strategy was adopted, the danger came to the family units from both competitors for food sources, and from predators (Wilson, 1975; Thorne et al., 2003). Early in evolution, termites thus evolved a caste of specialised defenders, the soldiers, which mostly fight with large mandibles and defensive glands (Deligne et al., 1981; Prestwich, 1984; Šobotník et al., 2010a). Termite soldiers can form up to 60% of foraging parties and 10% of the colony population, but can also completely disappear, as in many soil-feeding groups (Haverty, 1977).

Termites are formally split into “lower” termites, a paraphyletic assemblage of the basal groups, and “higher” termites containing the dominant family Termitidae, forming some 85% of the generic diversity (Krishna et al., 2013). Termitidae are further split into 8 subfamilies, with Apicotermitinae being always retrieved as a monophyletic group (Bourguignon et al., 2015, 2017; Buček et al., 2019; Romero Arias et al., 2021). All Apicotermitinae are believed to be soil-feeders, and the whole group reveals strong tendencies to spare the soldier caste, which was lost at least twice (Bourguignon

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et al., 2017; Buček et al., 2019; Romero Arias et al., 2021). When present, soldiers of Apicotermittinae are phragmotic (Deligne et al., 1981; Prestwich, 1984, J.Š. personal observation), i.e. the head is used to seal the galleries in order to prevent intruders to enter. In Apicotermittinae, soldiers are typically larger than workers, with heavily sclerotised head capsules and short and robust mandibles. The genus *Machadotermes* Weidner, 1974, is a member of the basal African soldiered groups with unknown phylogenetic position, with soldiers having the shortest mandibles of all Apicotermittinae studied so far. Deligne et al. (1981) and Quennedey, (1984) observed that the mandibles of the soldier in *Machadotermes inflatus* consist of two parts, the basal part being swollen, less sclerotized, and covered with numerous bristles and supposedly glandular openings (the so-called intramandibular gland), but nothing more is known.

One of the remarkable features of all social animals is their ability to communicate. Visual cues are of small importance to termites thus they depend before all on the chemical signals, with vibroacoustic communication playing an important part in alarm communication and group cohesion (Costa-Leonardo and Haifig, 2010; Bordereau and Pasteels, 2011; Delattre et al., 2019; Paillet et al., 2021). The classification of insect secretory cells follows the pioneering work of Noirot and Quennedey, (1974), who distinguished (i) class I secretory cells adjoining the cuticle and releasing their products directly to the exterior, (ii) class II cells being isolated from the cuticle by class I cells, which uptake their secretion and release it outside, and (iii) class III cells which are connected to the outside by cuticular canals supported by specialised cells. While the highest number of exocrine organs was described in social Hymenoptera (Billen and Šobotník, 2015), the most complex glands consisting of all classes of secretory cells were described only in cockroaches and termites (Ampion and Quennedey, 1981; Sreng, 1984, 1985; Quennedey, 1998; Šobotník and Hubert, 2003; Šobotník et al., 2003, 2005, 2015; Quennedey et al., 2008).

The exocrine glands are scattered all over the body, however, their functions remain in termites highly speculative apart of a few more thoroughly studied glands, such as (i) the sternal glands involved in the secretion of a trail pheromone and sometimes also a sex pheromone; (ii) the tergal glands and the posterior sternal glands known to produce exclusively a sex pheromone (Quennedey et al., 2004; Sillam-Dussès, 2010; Sillam-Dussès et al., 2005, 2011, 2020, 2021); (iii) the labial glands aiding several functions, such as food marking, phagostimulation, food digestion, feeding of dependent castes, building activities (all in workers), and defence (in all soldiers, and workers in some species as well; Noirot, 1969; Grassé, 1982; Reinhard et al., 2002; Tokuda et al., 2002; Fujita et al., 2008; Sillam-Dussès et al., 2012; Delattre et al., 2015); (iv) The frontal gland fully devoted to defence and occurring in soldiers and many imagines and workers of some advanced groups (Šobotník et al., 2010b, 2010c; Kotalová et al., 2013). While these glands are very common in most termite species, some others are unique to particular lineages, such as the defensive crystal glands involved in the self-sacrifice of old *Neocapritermes taracua* (Termitidae: Termitinae) workers (Šobotník et al., 2012; Bourguignon et al., 2016) or the defensive dehiscent organs in *Ruptitermes* (Termitidae: Apicotermittinae) workers (Costa-Leonardo, 2004; Costa-Leonardo et al., 2020; Poiani and Costa-Leonardo, 2016). Unfortunately, we do not have a satisfactory knowledge about the function of other glands known in termites.

The intramandibular glands were reported in many social Hymenoptera including ants (Schoeters and Billen, 1994), bees (Costa-Leonardo, 1978) and wasps (Penagos-Arévalo et al., 2015). The intramandibular glands always consist of class III secretory cells, however, their position and the localization of the excretory pores on the mandibles vary among particular taxa (Schoeters and Billen,

1994). The intramandibular glands in hymenopterans are supposed to play a role in nutrition (food capture or food processing), nest building, or production of a lubricant for the mandible joints (Nedel, 1960). Workers in the ant *Oecophylla* are known to rub their mandibles onto the substrate to recruit nestmates, probably by spreading the secretion of the intramandibular gland (Roux et al., 2010). The presence of the intramandibular gland in termites was hypothesized based on scanning electron microscopy of *M. inflatus* soldiers only (Deligne et al., 1981; Quennedey 1984).

We investigated the internal head structure of Apicotermittinae soldiers in order to address a hypothesis on the presence of a novel termite intramandibular gland in the genus *Machadotermes*, as well as in other members of the Apicotermittinae subfamily to test the phylogenetic distribution of the hypothetical gland. We also report here on the presence of other poorly studied cephalic glands in the Apicotermittinae soldiers.

2. Material and methods

Workers and soldiers of *Machadotermes rigidus* Collins, 1977 (Blattodea: Termitidae: Apicotermittinae) were, along with *Duplidentitermes furcatidens* (Sjöstedt, 1924) and *Jugositermes tuberculatus* Emerson, 1928, collected nearby Ebogo village (3.3940136235835454°, 11.471132614208443°, Mbalmayo area, Cameroon). Living termites were transported to Prague based on legal procedures, and all the terms and conditions of our work permits are met here (the sample use is based on terms and conditions specified in Permits N°010/MINRESI/B00/C00/C10/C12, N°075/MINRESI/B00/C00/C10/C12, N°079/CO/MINFOF/SETAT/SG/DFAP/SDVEF/SC/BJ, and N°079/P/MINFOF/SETAT/SG/DFAP/SDVEF/SC/BJ, all supervised by Dr. P. D. Akama). *Indotermes* sp. was collected in Xishuangbanna Tropical Botanical Garden, and kindly provided by Prof. Xiaodong Yang (Chinese Academy of Sciences).

Some soldiers of *M. rigidus* were stored in 80% ethanol and then dehydrated in 100% acetone for 2 days. Samples were dried by critical point CO₂, gold-coated, and observed using a Jeol 6380 LV scanning electron microscope (SEM). Living soldiers of *M. rigidus* were fixed in a 0.1 M phosphate buffer solution with 2% glutaraldehyde and 2.5% formaldehyde (both from Polysciences inc., EM Grade) for 1 day at +4 °C. The posterior parts of heads were removed in order to ease the fixative penetration, while the mouthparts were kept untouched. Otherwise, we used well-established procedures described in details by Šobotník et al. (2003) for subsequent steps. Semithin sections were stained with methylene blue and visualised using Nikon Eclipse Ni. Ultrathin sections were stained with uranyl acetate and lead citrate (standard recipe) and observed using a Jeol 1011 transmission electron microscope.

For the purpose of the micro-computed tomography (μCT) analyses, some soldier specimens of *M. rigidus*, *D. furcatidens*, *J. tuberculatus* and *Indotermes* sp. were preserved in 80% ethanol, and then they were stained for 48 h in absolute ethanol saturated with iodine. Each specimen was inserted into a plastic pipette tip filled with absolute ethanol and sealed with parafilm. μCT scans of specimens mounted on a rotating carousel were performed using a Zeiss Xradia 510 Versa 3D X-ray microscope and the Zeiss Scout-and-Scan Control System software (v11.1.6411.17883). 1601 projections were collected during 360° rotation of the specimens along an axis approximately corresponding to the longitudinal head capsule axis. For the overview of scanning parameters, see Supplementary Table 1. The 3D reconstructions of collected projections were performed with the Zeiss Scout-and-Scan Control System Reconstructor software (v11.1.6411.17883). In total, five scan datasets for four species were collected at a resolution of 1.5–3.9 μm. Virtual sections and visualizations of reconstructed

scan data were performed in Amira software (v6.7). The virtual longitudinal and transversal sections were reconstructed with the function “Orthoslice”. The colour space was globally adjusted to maximize visibility of morphological structures in both bright and dark spectra in the sections. The external body surface was visualized using the “Volume rendering” function by setting global colour space thresholds to values which maximized contrast between the specimen exoskeleton and the surrounding ethanol. All static images were rendered using the “Snapshot” function at maximal available resolution (1900 × 1200 pixels). Animation of sections traversing sagittally through the head capsule of *Machadotermes* were rendered using the “Animation” module in Amira software. Raw μ CT data are available upon request.

3. Results

The μ CT observations showed a prominent thickening of the epidermal cell layer inside of the mandible bases in soldiers of *M. rigidus* (Fig. 1C). The intramandibular gland occupies predominantly the outer (lateral) and partially ventral and dorsal parts of the mandible base (see Supplementary Video). The thickening was absent in soldiers of *Jugositermes* (Fig. 1D), *Duplidentitermes* (Fig. S8) and *Indotermes* (Fig. S12). Similarly to Deligne et al. (1981) and Quennedey (1984), SEM observations confirmed the presence of

numerous pores at the outer bases of mandibles in soldiers (Figs S1, S2), and their absence in workers (Figs S3, S4). Mandible bases in *M. rigidus* soldiers contained a clear epidermal thickening (Fig. 1B), which was made of class III secretory cells (Fig. 2). The gland was separated from the cuticle (thick between 20 and 25 μ m) by a layer of thin (200–500 nm) epidermal cells (Figs. 2B, S16, S17). The canals connecting the secretory cells to the exterior ran throughout the mandible cuticle (Fig. 2A). The unmodified epidermal cells underneath the cuticle, as well as the canal cells had condensed nuclei, small volume of cytoplasm, a few mitochondria and rough endoplasmic reticulum, attesting a low activity of the cells. In contrast, the secretory cells were much larger, between 10 and 15 μ m in length (axis parallel to the cuticle) and 4–10 μ m in width (axis perpendicular to the cuticle). The central parts of the secretory cells were occupied by a coiled end apparatus (or the extracellular reservoir) lined with spaced microvilli (about $1 \times 0.15 \mu$ m), among which the porous receiving canal (outer diameter around 650 nm, wall thickness 100–150 nm) was inserted. Closer to the cuticle, the receiving canal changed abruptly into the conducting canal, thick about 450 nm and consisting of epicuticular layers continuous with the mandible epicuticle. The conducting canals were made of the outer (20 nm thick) and the inner (80 nm thick) epicuticle. The structure of the conducting canals was conserved throughout their length, and although the inner epicuticle tended to get thinner inside of the

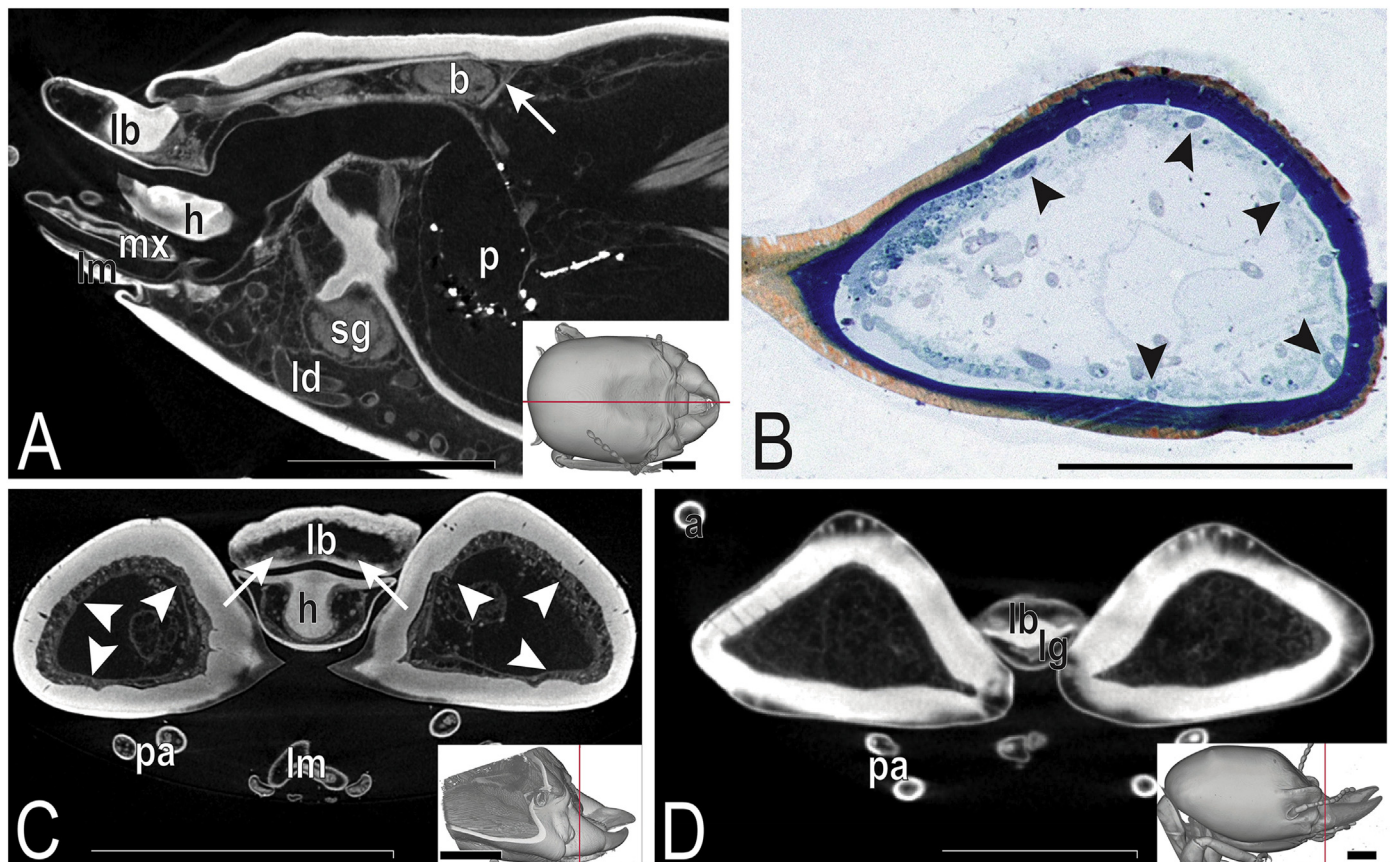


Fig. 1. Virtual micro-computed tomography sections through Apicotermitinae soldier heads (A, C, D), and optical microscopy of the intramandibular gland in *M. rigidus* (B). **A.** Sagittal section of *Machadotermes rigidus* soldier head; inset shows the section plane. Note that the labral gland is missing in the sagittal part of the head, as it appears as two regions located parasagittally as shown in Fig. 1C. The arrow marks the tentorial-fontanellar muscle. Scale bars represent 500 μ m. **B.** Transversal section of *Machadotermes rigidus* soldier mandible as shown by optical microscopy. Arrowheads mark the intramandibular gland. Scale bar represents 200 μ m. **C.** Transversal section through the mandible bases in *Machadotermes rigidus*. The intramandibular gland is placed amongst the arrowheads. Arrows mark the labral gland. Scale bars represent 500 μ m. **D.** Transversal section through the mandible bases in *Jugositermes tuberculatus* soldier. Note the absence of the intramandibular gland. Scale bars represent 500 μ m. **Abbreviations:** a, antenna; b, brain (supraesophageal ganglion); h, hypopharynx; lb, labrum; ld, labial gland ducts; lg, labral gland; lm, labium; mx, maxilla; p, pharynx; pa, palps (maxillary or labial); sg, subesophageal ganglion.

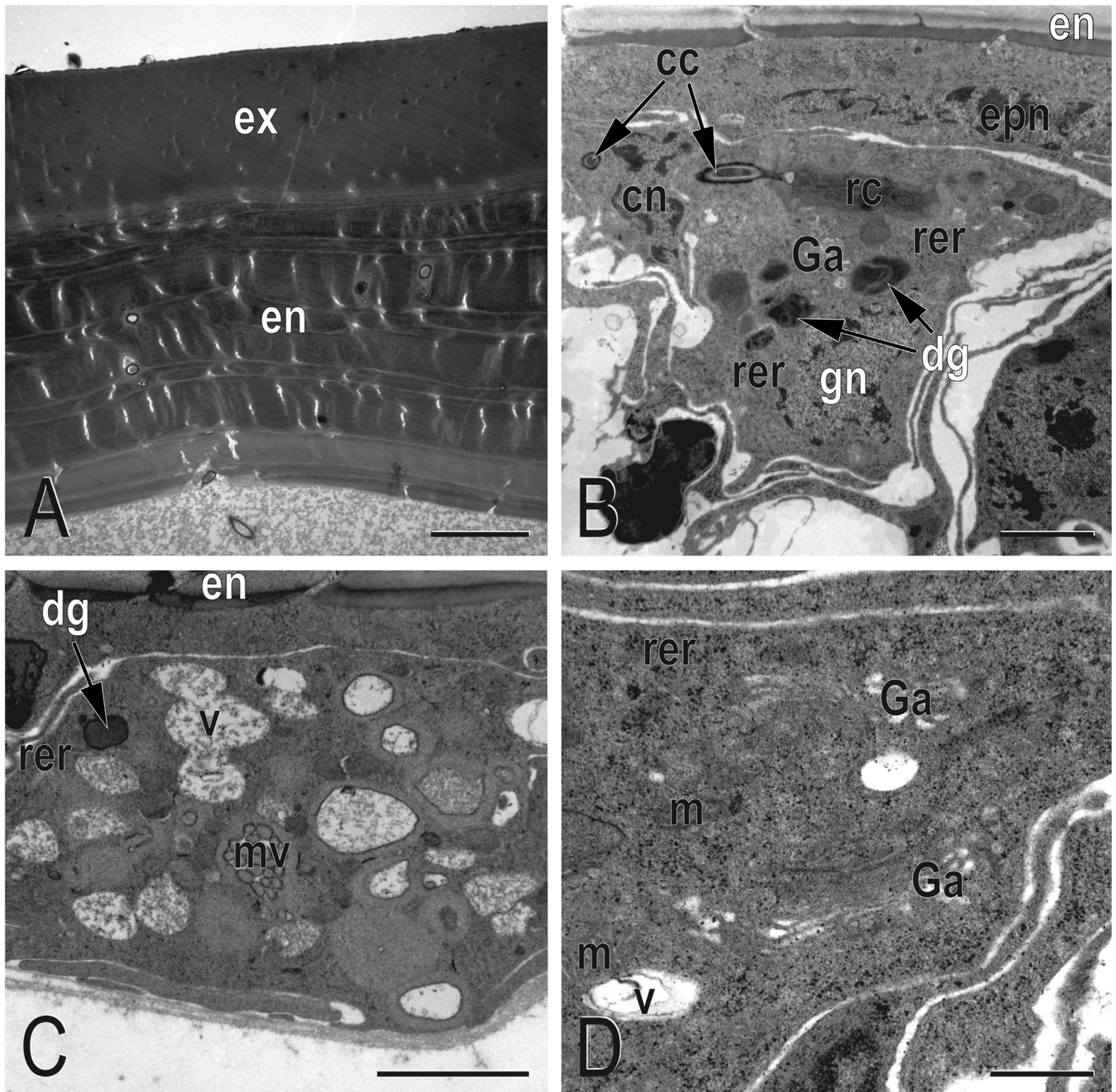


Fig. 2. Ultrastructure of the intramandibular gland in *Machadotermes rigidus*. **A.** Glandular cuticle with manifold canals of the gland cells. Scale bar represents 5 μm . **B.** Secretory cell showing nucleus, electron-dense granules, receiving and conducting canal. Scale bar represents 2 μm . **C.** Cytoplasm of a secretory cell showing vesicles in different stages of development. Scale bar represents 2 μm . **D.** Details of secretory organelles, rough endoplasmic reticulum and Golgi apparatus. Scale bar represents 500 nm. **Abbreviations:** cc, conducting canal; cn, nucleus of canal cell; dg, electron-dense granule; en, endocuticle; epn, nucleus of epidermal cell; ex, exocuticle; Ga, Golgi apparatus; gn, nucleus of glandular cell; m, mitochondrion; mv, microvilli; rer, rough endoplasmic reticulum; rc, receiving canal.

mandible wall, it was still clearly recognizable from the surrounding chitin cuticle. The other structures repeatedly observed in the mandible cuticle and underlying tissues were mechanoreceptive setae (with a single sensory axon; Fig. S18) with their supporting cells.

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.asd.2021.101136>

Each secretory cell contained on its periphery an irregular nucleus (roughly $6 \times 4 \mu\text{m}$) made of dispersed chromatin as well as

several chromatin aggregates. The secretory organelles comprised predominantly cisternal rough endoplasmic reticulum (RER) and Golgi apparatus. Elongated mitochondria (about 500 nm) were frequent but not abundant. The secretory inclusions were moderately abundant, but their presence was variable amongst the secretory cells. There were rather rare electron-dense granules, which decomposed within the cytoplasm through different ways (see Fig. 2C) and the final stages, vesicles (1–3 μm in diameter) of heterogeneous content (particles of units of nanometre size

dispersed in the electron-lucent matrix) were released at the microvilli bases. The basal parts of the secretory cells were simple, with a few shallow invaginations devoid of pinocytotic activity. The basement membrane was very thin, about 5 nm, and the different parts of the secretory cells layer were covered by 1–20 membrane layers. We did not observe any axon being eventually responsible for the gland innervation.

The μ CT scans of *Machadotermes*, *Duplidentitermes*, *Jugositermes*, and *Indotermes* soldier heads allowed us to confirm the general presence of the labral glands at the ventral labrum and dorsal hypopharynx (Figs S6, S8, S10, S12, S14), and of the mandibular glands that were small, located at the ventrolateral sides of the head (not shown). The frontal gland was missing completely in *Machadotermes*, as evidenced by the attachment of the fronto-tentorial muscle, which is attached to the frontal gland if present (Fig. 1A). At the same time, the frontal gland was highly reduced in *Indotermes* (Fig. S10), and occurred as a small sac in *Duplidentitermes* (round, about 300 μ m in diameter; Fig. S6) and *Jugositermes* (ovoid, roughly 200 \times 300 μ m; Fig. S14). We could also observe the ducts of the labial gland acini and reservoirs merging in the posteroventral head and opening at the base of labium (Fig. S10), but neither the acini nor reservoirs, which are presumably located in the thorax or anterior abdomen.

4. Discussion

Termite soldiers are a fascinating result of evolution driven by a plentitude of ecological variables. They are involved almost exclusively in colony defence, and the contribution of mandibles and defensive glands (mostly frontal, but also labial or novel, group-specific, glands) has been studied by several authors (for review see e.g. Noirot, 1969; Prestwich, 1984; Quennedey, 1984; Šobotník et al., 2010a). There are a series of cephalic glands that are broadly distributed, such as the frontal, mandibular or labial glands (Noirot, 1969; Deligne et al., 1981; Quennedey, 1984). At the same time, other glands were described only recently, although they occur across many termite groups, such as the clypeal, oral or labral gland (Křížková et al., 2014; Synek et al., 2019; Palma-Onetto et al., 2018, 2019). However, some of the cephalic glands reveal a narrow taxonomic distribution, such as the intramandibular gland found in *Machadotermes* soldiers, which was known only from older SEM observations (Deligne et al., 1981; Quennedey, 1984). The presence of this gland motivated our study of this rare, obscure and vulnerable termite. While we confirmed the existence of the intramandibular gland and described its ultrastructural details, one of the methods used, μ CT, allowed us to observe the inner anatomy of a series of soldiers' heads in four Apicotermite species. We can thus confirm that the intramandibular gland is an autapomorphy of the genus *Machadotermes*, while the frontal gland is variably developed, from missing in *Machadotermes*, tiny in *Indotermes* to small in *Duplidentitermes* and *Jugositermes* (see Fig. 1A, S6, S10, S14).

Most of Apicotermite species lost the soldier caste, which is retained in basal African taxa including *Machadotermes* as well as in the Asian Apicotermite clade, to which the genus *Indotermes* belongs (Bourguignon et al., 2017; Romero-Arias et al., 2021). The mandibles of Apicotermite soldiers always show two distinct parts - a somewhat swollen and less-sclerotised basal part with matting shagreen surface, and apical blades made of highly sclerotised, smooth and shiny cuticle. However, this pattern is even more pronounced in the genus *Machadotermes*, which also has the shortest mandibles among all Apicotermite soldiers. Moreover, the soldier ratio is surprisingly high in *Machadotermes* (roughly 1:10 soldier-to-worker ratio in *M. rigidus*, J.Š. personal observation) compared to all other Apicotermite, in which the soldiers,

if present, form usually less than 1% of the colony members (Haverty, 1977). In the extreme, soldiers were completely lost in two groups living in Africa and South America (Bourguignon et al., 2015, 2017; Romero-Arias et al., 2021). While soldiers are rare in many basal lineages (*Jugositermes*, *Coxotermes*, *Heimitermes*, *Phoxotermes*, *Duplidentitermes*, *Indotermes*), they are extremely rare in certain species of the genera *Eburnitermes* (J.Š. personal observation) and *Euhamitermes* (Haverty, 1977, J.Š. personal observation), in which there is not more than 1 soldier on roughly 50,000 workers, making the soldiers probably insignificant for the colony defence. In addition, Apicotermite soldiers and workers reveal in general rather short legs making them only slowly-moving animals well suited for soil-dwelling, but this is certainly not true for *Machadotermes* and two other genera, *Labidotermes* and *Acutidentitermes*, in which the sterile castes have long legs and are thus fast and agile, and their primary defence depends on their extraordinary ability to disappear fast from the sight of a predator (J.Š. personal observation).

With a few exceptions, the humivorous diet in Apicotermite favours nesting within the soil, most frequently in somewhat diffuse nests (Noirot, 1970; J.Š. and D.S.-D. personal observation). Interestingly, we found one *M. rigidus* colony including the queen, eggs, larvae, workers and soldiers moving freely in the leaf litter, without any nest, royal cell or even galleries. Instead of hiding inside the gallery system, the individuals were able to escape very fast, including the queen, in which hydrostatic movements took place due to physogastry. The high proportion of soldiers in comparison to the other Apicotermite may be explained by this nesting habit, the workers being virtually defenceless without a shelter to protect them. Even though the mandibles of the soldiers cannot inflict deep wounds, we may expect them to protect well the individuals of the colony with their phragmotic and well sclerotized heads in the dense network of leaf litter and roots. However, we cannot exclude that the colony we saw was in the process of migration from one nest to another, as already seen at least in the Neotropical *Anoplotermes banksi* and the Afrotropical *Amalotermes* sp. (J.Š. personal observation). This nesting habit, if confirmed, makes *Machadotermes* an extraordinary genus not only among Apicotermite, but also among all termite groups.

Our study confirmed the existence of an intramandibular gland in soldiers of *M. rigidus* made of class III secretory cells, and its absence in other inspected Apicotermite species. Glands made of class III cells are very common in insects, being scattered throughout the body, virtually from antennae tips through tarsi to the posterior abdomen. The secretory cells making up the intramandibular gland in *M. rigidus* soldiers are characterised by the presence of RER and Golgi apparatuses, and moderate amounts of electron-lucent secretory vesicles. Glands of the same ultrastructure are quite common in termites: e.g. posterior sternal glands in *Macrotermes annandalei* alate imagoes (Quennedey et al., 2004), tergal and posterior sternal glands in *Prorhinotermes simplex* alate imagoes (Šobotník et al., 2005), sternal glands of Rhinotermitidae workers (Quennedey et al., 2008), labral gland in *Glossotermes oculatus* soldiers (Šobotník et al., 2010d), crystal glands in *N. taracua* workers (Šobotník et al., 2014), clypeal gland in termite imagoes (Křížková et al., 2014), nasus gland in *Angularitermes coninasus* soldiers (Šobotník et al., 2015), and tarsal glands in several termite species (Costa-Leonardo et al., 2015). A very similar ultrastructure was observed also in the ventral scape gland in *Strumigenys* ants (Wang et al., 2021). On the other hand, many class III secretory cells differ in the ultrastructure, e.g. by dominance of smooth endoplasmic reticulum (SER), as in lateral thoracic glands of termites (Gonçalves et al., 2010), abdominal tegumental glands in stingless bees (Cruz-Landim et al., 2006), metasternal gland in the true bug *Rhodnius* (Lopes et al., 2020), and procoxal gland in *Discothyrea* ants

(Billen et al., 2020). Another feature not observed in *M. rigidus* is a high amount of electron-dense secretion as observed in the honeybee hypopharyngeal gland (Deseyn and Billen, 2005), leg glands in ants (Billen and Peeters, 2020), or spermathecal gland in scorpion fly (Yang and Hua, 2021). *P. simplex* neotenic reproductives display epidermal glands made also of class III cells but they are different as well, as class III secretory cells are huge and highly-active, producing enormous amounts of secretion by combined activity of RER and SER (Šobotník et al., 2003). A similar structure is observed in the antennal glands in bee males, but the heterogeneous secretion is produced without contribution of neither RER nor SER (Romani et al., 2003).

The function of the intramandibular gland was not resolved in the previous contributions (Deligne et al., 1981; Quennedey, 1984), and we can also just speculate about the gland function due to the material paucity. Deligne et al. (1981) and Quennedey (1984) suggested that, when soldiers detect an enemy, they lock their mandibles on the enemy and the intramandibular glands release a toxic secretion penetrating the wound through the furrows which run along the mandibles and facilitate the discharge of the secretions toward the mandibular blades. However, our study showed the absence of a gland reservoir and of neuronal innervation of the gland which excludes direct control over the secretion release, apparently essential for any defensive gland (Chapman, 2013). Thus, we assume that the intramandibular gland does not directly participate on the defensive activities, and other hypotheses should be considered. On one hand, during the collecting and sorting of the material, we have never observed a soldier of *M. rigidus* fighting an enemy, what is also unusual as the generalist ants usually show a strong interest into termites whenever spotted (J.Š. personal observation). In the case of *M. rigidus*, soldiers and also workers are rather avoided by the ants, suggesting the presence of a potent repellent signal, which can eventually originate from the intramandibular gland. Interestingly, our ultrastructural observations strongly suggest that the intramandibular gland produces a peptidic or proteinaceous secretion, not suitable for air-borne communication due to low volatility. Thus, based on the combined observations, we may hypothesize that the intramandibular gland secretion functions as a general protectant or antimicrobial agent, similar to notoriously known lysozymes of many different origins (Callewaert and Michiels, 2010), and spread through the colony by trophallaxis. The ultrastructural observations suggest that the secretion is produced and temporarily stored within the secretory cells in the form of electron-dense (highly probably proteinaceous) granules, which gradually dissolve into the secretory vesicles to be released later on, without direct control.

The μ CT scanning is a very efficient technique to compare the organisation of various tissues in insects and other small animals. For example, Quennedey (1984) referred to the presence of a frontal gland in *M. inflatus*, while it is clearly missing in *M. rigidus* (see Figs. 1A, S1, Supplementary video). Even though the gland is absent, the tentorial-fontanelle muscle is visible in *M. rigidus*, as well as in all termite species and developmental stages, irrespectively of the frontal gland development (Noirot, 1969; Grassé, 1982). The muscle is usually attached to the posterior frons (tip of the triangle), but when the frontal gland is developed, it stretches between the ventro-apical part of the frontal gland reservoir and tentorium, and presumably opens the frontal gland opening (fontanelle), when the secretion is pushed away by the contraction of mandibular or intersegmental muscles (Šobotník et al., 2010b; Kotalová et al., 2013). The frontal gland is on the other hand rather small but clearly present in the two other genera studied here, *Duplidentitermes* and *Jugositermes*. Lastly, the frontal gland seems to be present also in *Indotermes*, but its tiny size (see Fig. S10) makes its contribution to defensive activities doubtful. Our μ CT observations

also allowed us to confirm the presence of labral, mandibular and labial glands in all soldiers of the Apicotermatinae genera studied here (similarly to all other termites studied so far) but the details of their development were not obvious in μ CT scans.

Author statement

Tereza Beránková: material collecting, fixation, sectioning, electron microscopy, writing the first draft.

Aleš Buček: μ CT, extensive text edits.

Thomas Bourguignon: material collecting, text edits, graphic work.

Johanna Romero Arias: electron microscopy, text edits, graphic work.

Pierre D. Akama: responsible for permits, help with the field work.

David Sillam-Dussès: conceptualization, material collecting, extensive text edits.

Jan Šobotník: conceptualization, material collecting, extensive text edits, final text and revision preparation, acquisition of the research grants.

Acknowledgements

We thank the people of Ebogo II village (Cameroon) for their help and support during the field work. Prof. Xiaodong Yang (Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences) is greatly acknowledged for providing the material of Chinese termites. We are grateful to Rudi Scheffrahn (University of Florida) for the termite determination. We are grateful to Mirek Hylíš (Laboratory of Electron Microscopy, PFF UK) for his help with TEM. This study was supported by the Internal Grant Agency of the Faculty of Tropical AgriSciences CZU No. 20213112.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.asd.2021.101136>.

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