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Author	Jiri Synek, Tereza Berankova, Petr Stiblik, Jitka Pfliegerova, Pierre D. Akama, Thomas Bourguignon, David Sillam-Dusses, Jan Sobotnik
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The oral gland, a new exocrine organ of termites.

Jiří Synek^{a,*}, Tereza Beránková^{a,*}, Petr Stiblík^a, Jitka Pfliegerová^b, Pierre D. Akama^c, Thomas Bourguignon^{a,d}, David Sillam-Dussès^e, Jan Šobotník^{a,†}

^a Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Kamýcká 129, 165 21 Praha 6 Suchbát, Czech Republic

^b Institute of Entomology, Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

^c Département des Sciences Biologiques, Ecole Normale Supérieure, Université de Yaoundé I, BP 47 Yaoundé, Cameroon

^d Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Okinawa 904-0495, Japan

^e Université Paris 13 – Sorbonne Paris Cité, LEEC, EA 4443, 99 avenue Jean-Baptiste Clément, 93430 Villetaneuse, France

* Equal contribution

† Corresponding author: sobotnik@fld.czu.cz

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Abstract

Termites have a rich set of exocrine glands. These glands are located all over the body, appearing in the head, thorax, legs and abdomen. Here, we describe the oral gland, a new gland formed by no more than a few tens of Class I secretory cells. The gland is located on the dorsal and ventral side of the pharynx, just behind the mouth, and is divided into two regions. The dominant secretory organelle is smooth endoplasmic reticulum. Secretion release is under direct control of axons located within basal invaginations of the secretory cells. The

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secretion is released through modified porous cuticle located at the mouth opening. We confirmed the presence of the oral gland in workers and soldiers of several wood- and soil-feeding species of Rhinotermitidae and Termitidae, suggesting a broader distribution of the oral gland among termites. The oral gland is the smallest exocrine gland described in termites so far. We hypothesise that the oily secretion can either ease the passage of food or serve as a primer pheromone.

1. Introduction

Termites are the most abundant insects of tropical land biotopes (Engel et al. 2009; Bar-On et al. 2018). They are decomposers of dead plant material at various decomposition stages, starting from freshly dead plant tissues, and ending with humified remnants of organic matter scattered within inorganic soil matrices (Donovan et al. 2001; Bourguignon et al. 2011). The success of termites is due to a combination of factors, including their lignocellulose diet, their complex social structures and foraging strategies, and their lifestyle in closed defensible systems of nests and galleries allowing for advanced defensive and communication abilities (Šobotník et al. 2010a; Krishna et al. 2013).

Most termite activities are mediated by exocrine organs. As many as 20 exocrine glands have previously been described in termites (Šobotník et al. 2015), and there is a growing level of understanding about their involvement in defence. The frontal gland, used in defence, is perhaps the most studied organ of termites, and is found in soldiers, presoldiers, imagoes and some workers of Neoisoptera (Quennedey 1984; Šobotník et al. 2004, 2010a, b; Kotalová et al. 2013). The labial glands of soldiers always have defensive functions, while that of workers are usually involved in feeding activities (Noirot 1969; Sillam-Dussès et al. 2012). One notable exception is the labial glands of *Neocapritermes taracua*, which produce the precursors of a defensive secretion released by self-sacrifice (Šobotník et al. 2012; Bourguignon et al. 2016). Additional defensive glands have evolved in workers of several taxonomic groups, such as the dehiscent glands in *Ruptitermes* spp. (Poiani and Costa-Leonardo 2016), the mandibular glands in *Constrictotermes cyphergaster* (Costa-Leonardo and Shields 1990), and the crystal glands in *Neocapritermes taracua* (Šobotník et al. 2014).

Many exocrine glands are also known to be involved in communication. Such organs comprise the labral gland, helping to coordinate defensive activities (Palma-Onetto et al. 2018, 2019), the sternal gland, that secretes trail-following pheromones (Bordereau and Pasteels 2011; Sillam-Dussès 2010), the labial and frontal glands of soldiers, producing alarm pheromones in several species (Vrkoč et al. 1978; Pasteels and Bordereau 1998; Cristaldo et al. 2015; Delattre et al. 2015), and the imaginal sternal, posterior sternal and tergal glands, known to produce sex pheromones (Bordereau and Pasteels 2011; Sillam-Dussès et al. 2011). However, the function of most termite glands discovered so far remains hypothetical.

During our research on termite glands, we noticed the presence of a yet-unknown exocrine organ located at the boundary between the mouth and the pharynx, just behind the mouth opening. In this paper, we describe this new gland, and name it the “oral gland”. We selected three distant termite species, *Prorhinotermes simplex* (Rhinotermitidae), *Sphaerotermes sphaerothorax* (Termitidae: Sphaerotermitinae) and *Spinitermes trispinosus* (Termitidae: Termitinae), in which we report the presence of the oral gland, both in workers and soldiers.

2. Material and Methods

Termite material

We used termite colonies maintained in breeds in Prague, Czech Republic. *Prorhinotermes simplex* (Hagen, 1858) is kept in laboratory breeds since it was collected in Soroa, Piñar del Rio (Cuba) in 1964; *Sphaerotermes sphaerothorax* (Sjöstedt, 1911) was imported, with legal permits, from Ebogo II, Mbalmayo (Cameroon), in 2016; *Spinitermes trispinosus* (Hagen and Bates, 1858) was imported, following on legal procedures, from Petit Saut (French Guiana, France) in 2016.

Microscopy procedures

Living termites (workers and soldiers) were fixed in a 0.1M phosphate buffer solution with 2% glutaraldehyde and 2.5% formaldehyde (both from Polysciences inc., EM Grade) for 1 day at room temperature. The mandibles were carefully removed to ease subsequent sectioning. We

followed the procedure described in detail by Šobotník et al. (2004) 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.

3. Results

In termites, the dorsal part of the preoral cavity (i.e. the cibarium) opens into the pharynx. The pharynx, located posterior to the mouth, is oval-shaped in cross section. The oral gland was composed of two groups of secretory cells located at the mid part of the dorsal and ventral pharynx (Fig. 1A). The gland consisted of 30 to 60 secretory cells. As the basic structure of the gland remained unchanged among all studied species and castes (see Figs 1 and S1), the following description summarises the common characteristics. Specific features are listed at the end of the Results section. The main characteristics of the oral gland were most obvious in the gland centre, while the ultrastructure of peripheral cells gradually changed to resemble that of unmodified epithelium. The cells located near the secretory epithelium comprised: (i) standard epidermal cells with a few organelles and nuclei rich in condensed chromatin, (ii) epidermal cells attaching the muscles to the pharynx cuticle through numerous microtubules, located inside the cells, and tonofibrillae, embedded into the cuticle matrix outside the cells, and (iii) sparse chemoreceptors containing four or five dendrites (not shown).

The secretory epithelium was formed exclusively by Class I secretory cells (*sensu* Noirot and Quennedey 1974). The epithelium was between 5 and 10 μm thick, both on the dorsal and ventral faces of the anterior pharynx. The secretory cells were cubic, or slightly columnar, and contained irregular nuclei (about 4 μm in the largest dimension) located at cell bases, and filled predominantly with dispersed chromatin interspersed with larger chromatin aggregates (Figs 1B, C). The secretory epithelium was covered by single- or multiple-layered basement membrane, roughly 40 nm thick. The epithelium located nearby muscle attachments was often markedly thicker, up to 200 nm in thickness, and contained collagen fibres. Basal invaginations were well-developed, although they never reached deeper than approximately one-third of the secretory epithelium thickness, and they only rarely revealed pinocytotic activity. Singular free axons were occasionally observed within the basement invaginations

(Fig. S2D). The cytoplasm at the secretory cell bases contained high amount of smooth Endoplasmic Reticulum (ER hereafter), made of roughly 50 nm thick tubules, and low amounts of rough ER, free ribosomes, electron-lucent vesicles, and occasionally free lipid-like droplets and protein granules surrounded by plasma membrane.

The apical portion of secretory cells included abundant smooth ER, abundant small mitochondria (about 0.5 μm in their longest dimension) and well-developed microvilli, about 100 nm thick and up to 0.6 μm long (Fig. 1D), the length and abundance of which decreased towards the gland periphery. The microvilli always possessed a central channel (Fig. S2B), thought to be connected to smooth ER, according to Quennedey (1998). Small lucent vesicles (about 500 nm in diameter) were often observed, and rarely seen to fuse with the apical plasma membrane at the microvilli bases (Fig. 1D). The intercellular junctions comprised apical zonula adherens, always followed by a septate junction (about 25 nm thick) and a gap junction (about 15 nm thick). The junctions between neighbouring secretory cells were approximately confined to the apical half, the rest of the cell membranes being devoid of junctions. The cuticle overlying the oral gland was formed by modified endocuticle saturating the secretion release. Endocuticle modifications varied with position, starting from enlarged pore canals and ending with endocuticle having a spongy appearance. The endocuticle was slightly thinner in workers (ranging, according to species, between 0.6 and 1 μm in thickness) compared to soldiers (1.4 to 1.9 μm thick, according to species) (compare Figs 1B, S1A, E, F). The exocuticle was relatively thin, penetrated by numerous pore channels leading to epicuticular pores piercing a very thin epicuticle (Fig. S2A). The thinnest exocuticle was observed in *S. sphaerotherax* (about 100 nm in both worker and soldier castes), followed by *P. simplex* (about 400 nm in worker and 550 nm in soldier), and the thickest exocuticle was found in *S. trispinosus* (about 400 nm in worker and 1 μm in soldier). The epicuticle was made of an inner epicuticle between 20 and 40 nm thick, and an outer epicuticle about 15 nm thick. The diameter of epicuticular pores was roughly 20 nm.

Apart from the general structure of the oral gland described above, *P. simplex* workers and soldiers possessed many glycogen granules, especially at the secretory cell bases. Both castes of *S. sphaerotherax* contained higher amounts of lipid-like droplets, especially in the ventral portion of the gland in workers. Another organelle present only in *S. sphaerotherax* is the Golgi apparatus, observed in rare cases at the secretory cell bases (Fig. S2C). Lastly, some of the oral

gland secretory cells found in *S. trispinosus* contained large lucent vesicles, up to 5 μm in diameter.

4. Discussion

Here we report the presence and ultrastructure of the oral gland, a new exocrine organ in termites. The gland was observed in both workers and soldiers of the wood-feeding termites *Prorhinotermes simplex* (Rhinotermitidae) and *Sphaerotermes sphaerothorax* (Termitidae: Sphaerotermatinae), and in the soil-feeding species *Spinitermes trispinosus* (Termitidae: Termitinae). The presence of the gland in unrelated termite species suggests it is widespread among termites. The oral gland is extraordinarily small, the smallest of all termite glands identified so far, which likely explains why it has been overlooked until now. At the same time, its universal distribution suggests it has an important function.

The glands of termites can be classified into two categories according to their distribution among castes and species. The glands present in all taxa and castes are the labral, mandibular, labial and sternal glands (Lambinet 1959; Noirot 1969; Šobotník and Hubert 2003; Šobotník and Weyda 2003; Palma-Onetto et al. 2018, 2019). All other glands are confined to some groups and/or castes (Billen and Šobotník 2015), with the exception of the frontal gland, which occurs in most castes in most species of Neoisoptera (Deligne et al. 1981; Quennedey 1984; Šobotník et al. 2010b; Kotalová et al. 2013; pers. observ.). The oral gland probably belongs to the former category, as it likely occurs in both workers and soldiers of “lower” (all families except Termitidae) and “higher” termites (only Termitidae), irrespectively of their diet, i.e. wood- or soil-feeders. This opinion is also supported by a single observation of the oral gland in a soldier of the wood-feeding termite, *Microcerotermes* sp. (Termitidae: Termitinae) (see Fig. S1F). Future studies are needed to confirm this statement.

The function of the oral gland is unclear, and we were unable to characterize it in the current study due to methodological constraints. However, we can formulate some hypotheses based on the structure and location of the gland. The cytoplasm of the secretory cells is dominated by smooth ER, an organelle known to produce lipidic substances that are often used for communication purposes (for review see Percy-Cunningham and MacDonald 1987 or Tillman et al. 1999). The production of lipid-like secretion is further corroborated by the presence of

droplets located freely in the cytoplasm in several samples studied here. Other secretory organelles observed within the secretory cells, such as rough ER or Golgi apparatus, reach considerably lower abundance and probably serve only for maintaining the secretory function achieved by the smooth ER. However, the position of the oral gland suggests its secretions are not used for communication, as the secretion is released just behind the mouth. Therefore, we suggest that the secretion may lubricate the entrance of the digestive tube, facilitating the passage of food particles (Donovan et al. 2001). Alternatively, the gland may produce the primer pheromones regulating caste development within colony. Indeed, termite colonies are usually founded by a couple of dealate imagoes, i.e. the future king and queen (called primary reproductives), and the formation of secondary reproductives (also called neotenics) is inhibited by the royal couple via a substance distributed by contact among nestmates (Lüscher 1961; Springhetti 1972). Some volatile substances playing a role in these inhibitory processes have recently been identified (Matsuura et al. 2010; Funaro et al. 2018), and could possibly be produced by the oral gland. The same is true for soldiers, which have an inhibitory effect on the formation of additional soldiers (Lefeuvre and Bordereau 1984; Mitaka et al. 2017). The presence of neotenics is very common in termites, especially in “lower” termites, and known to occur in two of the studied genera: *Protrichotermes* and *Microcerotermes* (Myles 1999). Thus, the primer pheromone could be released by the oral gland and then transmitted to other colony members during oral trophallaxis (McMahan 1969), as has already been suggested (Lüscher 1961). Other possible functions, such as production of releaser pheromones, digestive enzymes, or defensive compounds, seem quite unlikely due to the gland size, structure and ultrastructure.

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Appendix A. Supplementary data

References

- Bar-On, Y.M., Phillips, R. and Milo R., 2018. The biomass distribution on Earth. P. Natl. Acad. Sci. USA 115, 6506-6511.
- Billen, J. and Šobotník, J., 2015. Insect exocrine glands. Arthropod Struct. Dev. 44, 399-400.
- Bordereau, C. and Pasteels, J.M., 2011. Pheromones and chemical ecology of dispersal and foraging in termites. In: Bignell, D.E., Roisin, Y. and Lo N. (Eds.), Biology of Termites: A Modern Synthesis. Springer, Dordrecht, pp. 279-320.
- Bourguignon, T., Šobotník, J., Lepoint, G., Martin, J.M., Hardy, O.J., Dejean, A. and Roisin, Y., 2011. Feeding ecology and phylogenetic structure of a complex Neotropical termite assemblage, revealed by nitrogen stable isotope ratios. Ecol. Entomol. 36, 261-269.
- Bourguignon, T., Šobotník, J., Brabcová, J., Sillam-Dussès, D., Buček, A., Krasulová, J., Vytisková, B., Demianová, Z., Mareš, M., Roisin, Y. and Vogel, H., 2016. Molecular mechanism of the two-component suicidal weapon of *Neocapritermes taracua* old workers. Mol. Biol. Evol. 33, 809-819.
- Costa-Leonardo, A.M. and Shields, K.S., 1990. Morphology of the mandibular glands in pseudergates of *Constrictotermes cyphergaster* (Silvestri) (Isoptera: Termitidae). Int. J. Insect. Morphol. 19, 61-64.
- Cristaldo, P.F., DeSouza, O., Krasulová, J., Jirošová, A., Kotalová, K., Lima, E.R., Šobotník, J. and Sillam-Dussès, D., 2014. Mutual use of trail-following chemical cues by a termite host and its inquiline. PLOS One 9, e85315.

Delattre, O., Sillam-Dussès, D., Jandák, V., Brothánek, M., Rücker, K., Bourguignon, T., Vytisková, B., Cvačka, J., Jiříček, O. and Šobotník, J., 2015. Complex alarm strategy in the most basal termite species. *Behav. Ecol. Sociobiol.* 69, 1945-1955.

Deligne, J., Quennedey, A. and Blum, M.S., 1981. The enemies and defense mechanisms of termites. In: Hermann, H.R. (Ed.), *Social insects*, vol. II. Academic Press, New York, pp. 1-76.

Donovan, S.E., Eggleton, P. and Bignell, D.E., 2001. Gut content analysis and a new feeding group classification of termites. *Ecol. Entomol.* 26, 356-366.

Engel, M.S., Grimaldi, D.A. and Krishna, K., 2009. Termites (Isoptera): Their phylogeny, classification, and rise to ecological dominance. *Am. Mus. Novit.* 3650, 1-27.

Funaro, C.F., Böröczky, K., Vargo, E.L. and Schal, C., 2018. Identification of a queen and king recognition pheromone in the subterranean termite *Reticulitermes flavipes*. *P. Natl. Acad. Sci. USA* 115, 3888-3893.

Krishna, K., Grimaldi, D.A., Krishna, V. and Engel, M.S., 2013. Treatise on the Isoptera of the world. *Bull. Am. Mus. Nat. Hist.* 377, 1-2704.

Kotalová, K., Hanus, R., Bourguignon, T., Roisin, Y. and Šobotník, J., 2013. Armed reproductives: Evolution of the frontal gland in imagoes of Termitidae. *Arthropod Struct. Dev.* 42, 339-348.

Lambinet, F., 1959. La glande mandibulaire du termite à cou jaune (*Calotermes flavicollis*). *Insectes Soc.* 6, 165–177.

Lefeuvre, P. and Bordereau, C., 1984. Soldier formation regulated by a primer pheromone from the soldier frontal gland in a higher termite, *Nasutitermes lujae*. *P. Natl. Acad. Sci. USA* 81, 7665–7668.

Lüscher, M., 1961. Social control of polymorphism in termites. In: Kennedy, J.S. (Ed.), *Insect polymorphism*. Royal Entomological Society of London, pp. 57-67.

Matsuura, K., Himuro, C., Yokoi, T., Yamamoto, Y., Vargo, E.L. and Keller, L., 2010. Identification of a pheromone regulating caste differentiation in termites. *P. Natl. Acad. Sci. USA* 107, 12963–12968.

McMahan, E.A., 1969. Feeding relationships and radioactive techniques. In: Krishna, K. and Weesner, F.M. (Eds), *Biology of termites*, vol. I. Academic press, New York, pp. 387-406.

Mitaka, Y., Mori, N. and Matsuura, K., 2017. Multi-functional roles of a soldier-specific volatile as a worker arrestant, primer pheromone and an antimicrobial agent in a termite. *Proc. R. Soc. Lond., B, Biol. Sci.* 284, 20171134.

Myles, T.G., 1999. Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology* 33, 1-91.

Noirot, C., 1969. Glands and secretions. In: Krishna, K. and Weesner, F.M. (Eds), *Biology of termites*, vol. I. Academic press, New York, pp. 89-123.

Noirot, C. and Quennedey, A., 1974. Fine structure of insect epidermal glands. *Annu. Rev. Entomol.* 19, 61-80.

Palma-Onetto, V., Hošková, K., Křížková, B., Krejčířová, R., Pfliegerová, J., Bubeníčková, F., Plarre, R., Dahlsjö, C.A.L., Synek, J., Bourguignon, T., Sillam-Dussès, D. and Šobotník, J., 2018. Labral gland in termite soldiers. *Biol. J. Linn. Soc.* 123, 535-544.

Palma-Onetto, V., Pfliegerová, J., Plarre, R., Synek, J., Cvačka, J., Sillam-Dussès, D. and Šobotník, J., 2019. The labral gland in termites: Evolution and function. *Biol. J. Linn. Soc.* 126, 587-597.

Pasteels, J.M. and Bordereau, C., 1998. Releaser pheromones in termites. In: Van der Meer, R.K., Breed, M.D., Espelie, K.E. and Winston, M.L. (Eds.), *Pheromone Communication in Social Insects: Ants, Wasps, Bees and Termites*. Westview, Boulder, pp. 193-215.

Percy-Cunningham, J.E. and MacDonald, J.A., 1987. Biology and ultrastructure of sex pheromone-producing glands. In: Prestwich, G.D. and Blomquist, G.J. (Eds.). *Pheromone biochemistry*. Academic Press, London, pp. 27-75.

Poiani, S.B. and Costa-Leonardo, A.M., 2016. Dehiscent organs used for defensive behavior of kamikaze termites of the genus *Ruptitermes* (Termitidae, Apicotermitinae) are not glands. *Micron* 82, 63-73.

Quennedey, A., 1984. Morphology and ultrastructure of termite defense glands. In: Hermann, H.R. (Ed), *Defensive Mechanisms in Social Insects*. Praeger, New York, pp. 151-200.

Quennedey, A., 1998. Insect epidermal gland cells: Ultrastructure and morphogenesis. In: Harrison, F.E. and Locke, M. (Eds). *Microscopic Anatomy of Invertebrates*, vol. 11A. Wiley-Liss, New York, pp. 177-207.

Sillam-Dussès D., 2010. Trail pheromones and sex pheromones in termites. New York, Novinka, 79 pp.

Sillam-Dussès, D., Hanus, R., Abd El-Latif, A.O., Jiroš, P., Krasulová, J., Kalinová, B., Cvačka, J., Valterová, I. and Šobotník, J., 2011. Sex pheromone and trail pheromone of the sand termite *Psammotermes hybostoma* (Rhinotermitidae: Psammotermitinae). *J. Chem. Ecol.* 37, 179-188.

Sillam-Dussès, D., Krasulová, J., Vrkoslav, V., Pytelková, J., Cvačka, J., Kutalová, K., Bourguignon, T., Miura, T. and Šobotník, J., 2012. Comparative study of the labial glands secretion in termites (Isoptera) *PLOS One* 7, e46431.

Šobotník, J. and Hubert, J., 2003. The morphology of the exocrine glands of *Prorhinotermes simplex* (Isoptera: Rhinotermitidae) and their ontogenetical aspects. *Acta Societatis Zoologicae Bohemicae* 67, 83-98.

Šobotník, J. and Weyda, F., 2003. Ultrastructural ontogeny of the labial gland apparatus in *Prorhinotermes simplex* (Isoptera: Rhinotermitidae). *Arthropod Struct. Dev.* 31, 255-270.

Šobotník, J., Weyda, F., Hanus, R., Kyjaková, P. and Doubský, J., 2004. Ultrastructure of the frontal gland in *Prorhinotermes simplex* (Isoptera: Rhinotermitidae) and quantity of the defensive substance. *Eur. J. Entomol.* 101, 153-163.

Šobotník, J., Jirošová, A. and Hanus, R., 2010a. Chemical warfare in termites. *J. Insect. Physiol.* 56, 1012-1021.

Šobotník, J., Sillam-Dussès, D., Weyda, F., Dejean, A., Roisin, Y., Hanus, R. and Bourguignon, T., 2010b. The frontal gland in workers of Neotropical soldierless termites. *Naturwissenschaften* 97, 495-503.

Šobotník, J., Bourguignon, T., Hanus, R., Demianová, Z., Pytelková, J., Mareš, M., Foltýnová, P., Preisler, J., Cvačka, J., Krasulová, J. and Roisin, Y., 2012. Explosive backpacks in old termite workers. *Science* 337, 436.

Šobotník, J., Kotalová, K., Vytisková, B., Roisin, Y. and Bourguignon, T., 2014. Age-dependent changes in ultrastructure of the defensive glands of *Neocapritermes taracua* workers (Isoptera, Termitidae). *Arthropod Struct. Dev.* 43, 205-210.

Šobotník, J., Bourguignon, T., Carrijo, T.F., Bordereau, C., Robert, A., Křížková, B., Constantini, J.P. and Canello, E.M., 2015. The nasus gland: A new gland of *Angularitermes* (Termitidae, Nasutitermitinae). *Arthropod Struct. Dev.* 44, 401-406.

Springhetti, A., 1972. I reali nella differenziazione delle caste di *Kalotermes flavicollis* (Fabr.) (Isoptera). *B. Zool.* 39, 83-87.

Tillman, J.A., Seybold, S.J., Jurenka, R.A. and Blomquist, G.J., 1999. Insect pheromones - an overview of biosynthesis and endocrine regulation. *Insect. Biochem. Molec.* 29, 481-514.

Vrkoč, J., Křeček, J. and Hrdý, I., 1978. Monoterpenic alarm pheromones in two *Nasutitermes* species. *Acta. Entomol. Bohemos.* 75, 1-8.

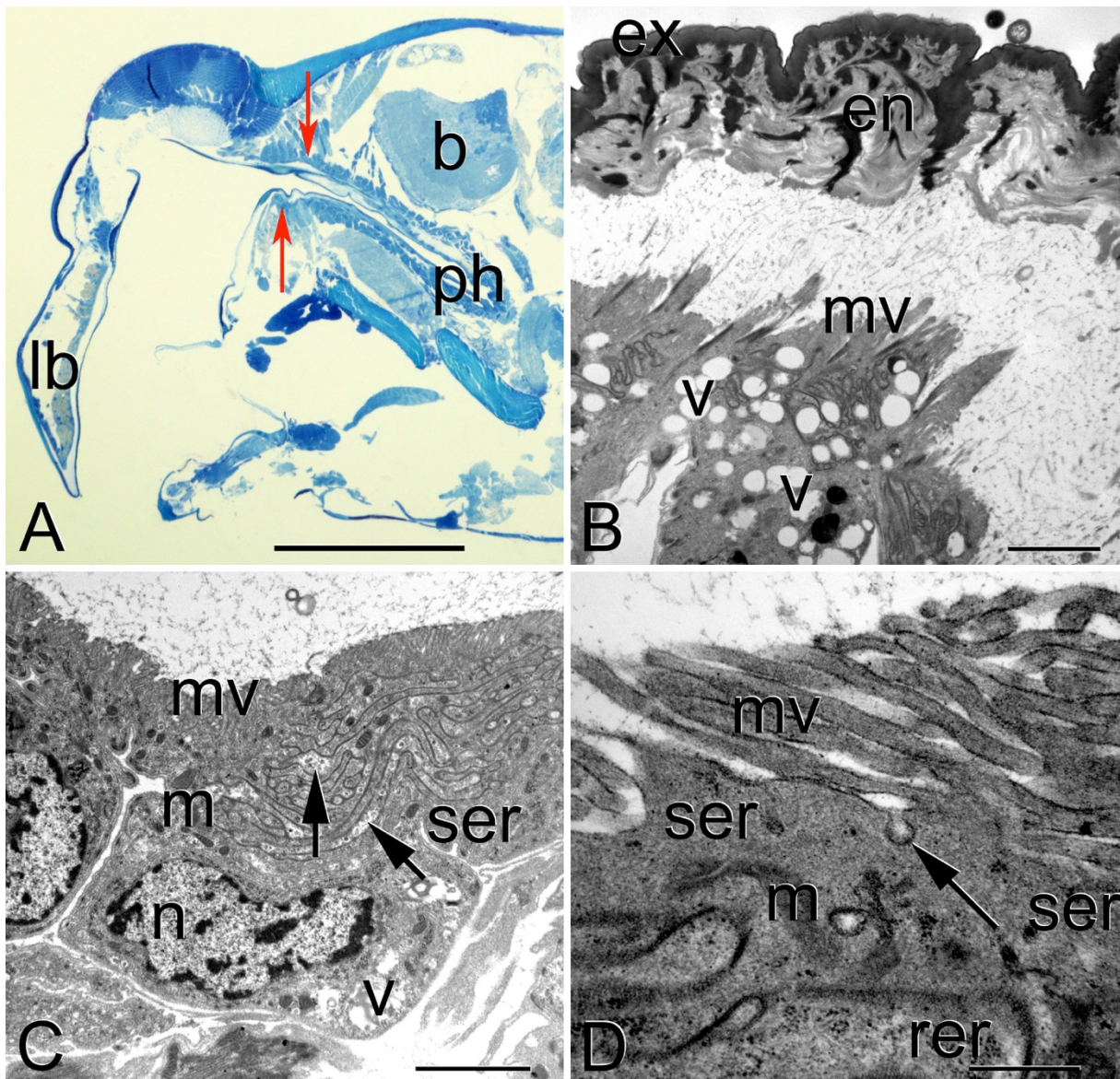


Figure 1: Development of the oral gland. A. Sagittal section of the head of *Sphaerotermes sphaerothorax* worker. Arrows mark the two regions of the oral gland secretory epithelium. Scale bar represents 200 μm . B. Overall development of the oral gland in *Spinitermes trispinosus* worker. Note the highly modified cuticle with numerous epicuticular pores. Scale bar represents 2 μm . C. The oral gland secretory epithelium in *Spinitermes trispinosus* soldier. The arrows mark glycogen granules. Scale bar represents 2 μm . D. A detail of the apical portion of the oral gland secretory cell in *Sphaerotermes sphaerothorax* worker. The arrow marks fusion of small secretory vesicle with apical membrane. Scale bar represents 0.5 μm . Abbreviations: b, brain (supraoesophageal ganglion); en, endocuticle; ex, exocuticle; lb, labrum; m, mitochondria; mv, microvilli; n, nucleus; ph, pharynx; rer, rough endoplasmic reticulum; ser, smooth endoplasmic reticulum; v, vesicles.