Czech University of Life Sciences Prague

Faculty of Forestry and Wood Sciences

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Rostral gland in soldiers of *Verrucositermes* sp. (Termitidae: Nasutitermitinae)

Diploma thesis

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Thesis title

Rostral gland in soldiers of Verrucositermes sp. (Termitidae: Nasutitermitinae)

Objectives of thesis

The aim of this work is to confirm the nature of the tubercles occurring on the head of soldiers of Verrucositermes sp. (Termitidae: Nasutitermitinae). These structures were described to be connected to yet-unknown exocrine organ called rostral gland based on scanning electron microscopy observation, however, the nature of this tissue was never confirmed. We will employ techniques of optical and electron microscopy to reveal what is the structure of this peculiar organ.

Methodology

Fixation of soldiers and workers of Verrucositermes sp. (Termitidae: Nasutitermitinae)

Scanning electron microscopy of soldier and worker heads and detailed analysis of the tubercles structure

Semithin sectioning of 3 soldier heads

Analysis of the soldier head tissues by means of optical microscopy

Ultrathin sectioning of 3 soldier heads

Analysis of the soldier head tissues by means of transmission electron microscopy

Basic measurements of tissues connected to the tubercles aiming at proving or disproving its glandular nature.

Preparation of results and graphic representation of the rostral gland, and composing a manuscript to be submitted to journal Arthropod Structure and Development

The proposed extent of the thesis

60 pages

Keywords

Termites, Isoptera, Blattodea: Termitoidae; cephalic exocrine glands

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Recommended information sources

Deligne J. 1983: Description, développement et af finités de Verrucositernies hirtus sp. n. Fonction glandulaire des tubercules du soldat (Isopteres Nasutitermitinae). Rev. Zool. Afr. 97: 533-548.

Křížková B., Bourguignon T., Vytisková B. & Šobotník J. 2014: The clypeal gland: a new exocrine gland in termite imagoes (Isoptera: Serritermitidae, Rhinotermitidae, Termitidae). Arthropod Structure & Development 43: 537-542.

Kutalová K., Hanus R., Bourguignon T., Roisin Y. & Šobotník J. 2013: Armed reproductives: Evolution of the frontal gland in imagoes of Termitidae. Arthropod Structure & Development 42: 339-348.

Palma-Onetto V., Hošková K., Křížková B., Krejčířová R., Pflegerová J., Bubeníčková F., Plarre R., Dahlsjö C.A.L., Synek J., Bourguignon T., Sillam-Dussès D. & Šobotník J. 2018: Labral gland in termite soldiers. Biological Journal of the Linnean Society 123: 535-544.

Piskorski R., Hanus R., Kalinová B., Valterová I., Křeček J., Bourguignon T., Roisin Y. & Šobotník J. 2009: Temporal and geographic variations in the morphology and chemical composition of the frontal gland in imagoes of Prorhinotermes species (Isoptera: Rhinotermitidae). Biological Journal of the Linnean Society 98: 384-392.

Synek J., Beránková T., Stiblík P., Pflegerová J, Akama P.D., Bourguignon T., Sillam-Dussès D & Šobotník J. 2019: The oral gland, a new exocrine organ of termites. Arthropod Structure & Development 51: 32-36.

Šobotník J., Bourguignon T., Carrijo T.F., Bordereau C., Robert A., Křížková B., Constantini J.P. & Cancello E.M. 2015: The nasus gland: A new gland of Angularitermes (Termitidae, Nasutitermitinae). Arthropod Structure & Development 44: 401-406.

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

Šobotník J., Bourguignon T., Hanus R., Sillam- Dussès D., Pflegerová J., Weyda F., Kutalová K., Vytisková B. & Roisin Y. 2010: Not only soldiers have weapons: Evolution of the frontal gland in imagoes of the termite families Rhinotermitidae and Serritermitidae. PLoS One 5: e15761. (doi:10.1371/journal.pone.0015761)

Šobotník J., Kutalová K., Vytisková B., Roisin Y. & Bourguignon T. 2014: Age-dependent changes in ultrastructure of the defensive glands of Neocapritermes taracua workers (Isoptera, Termitidae). Arthropod Structure & Development 43: 205-210.

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I declare that this thesis entitled Rostral gland in soldiers of *Verrucositermes* sp. (Termitidae: Nasutitermitinae) was developed independently by me under the leadership of doc. Mgr. Jan Šobotník Ph.D. All the sources have been quoted and acknowledged by means of complete references. I am aware by the publication of this thesis I agree to its publication in according with Act No. 111/1998 Coll. about universities, as emended, and regardless of the outcome of its defence.

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Bc. Alena Füllsacková

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Abstract

Termites (Dictyoptera: Isoptera; Blattodea: Termitoidae) belong to the most abundant animals in tropical ecosystem and they have colonised many habitats. There are roughly 3000 termite species described so far. Termites are known for their sheer abundance, pest statues and exceptional decomposing ability. The dead plant material is decomposed by termites and their allied symbiotic microorganisms. All termites are social insect with caste system and division of labour. Their communication is mostly based on glandular secretions. Termites possess 23 described glands so far. A variety functions has been described, production of pheromones, source of digestive enzymes or production of defensive chemicals. In following work, we are confirming glandular nature of the tubercles occurring on the head of soldiers *Verrucositermes tuberosus* (Termitidae: Nasutitermitinae). These structures were described to be connected to exocrine organ called rostral gland based on scanning electron microscopy observation. We employed techniques of optical and electron microscopy and found an exocrine gland consisting of numerous secretory cells, each connected to the exterior by a canal supported by a specialised cell (class III secretory cells). While the secretory cells are rich in organelles such as mitochondria, Golgi apparatus or glycogen, canal cells are virtually inactive.

Key words: Termites, Isoptera, Blattodea: Termitoidae, cephalic exocrine glands, rostral gland, microscopy

Abstrakt

Termiti (Dictyoptera: Isoptera; Blattodea: Termitoidae) tvoří jednu z nejpočetnějších skupin bezobratlých v tropických oblastech, kde mohou tvořit až 80 % biomasy. V současnosti je popsáno přibližně 3000 druhů a každý rok přibývají další. Termiti nejsou příliš prozkoumaná skupina. Pozornosti se jim dostalo především jako škůdcům, jsou ale především významní ekosystémoví inženýři a zásadně se podílejí na rozkladu rostlinné hmoty a cyklech živin. Je to eusociální hmyz, který je známý komplexními sociálními vztahy a výstavbou hnízd dosahujících obřích rozměrů. Komunikace mezi jedinci probíhá primárně přes sekreci exokrinních žláz. Celkem bylo doposud u termitů popsáno 23 exokrinních žláz, které mají rozmanité funkce např.: produkce feromonů, zdroj trávicích enzymů nebo produkce obranných látek. Následující práce obsahuje shrnutí termití biologie, vysvětlení jejich sociálních vztahů a popis známých a významných žláz. Praktická část se zabývá popsáním nově objevené rostrální žlázy v hlavách vojáků *Verrucositermes tuberosus* (Termitidae: Nasutitermitinae). Tato žláza je spojena s bradavičnatými výrůstky na jejich hlavách. Žláza byla zkoumána za pomocí optické a elektronové mikroskopie. Žláza je tvořena sekrečními buňkami III. třídy, které jsou spojeny s vnější prostředím kanálkovou buňkou. Sekreční buňky byly pozorovány jako aktivní s velkým množstvím organel a kanálkové buňky jako inaktivní.

Klíčová slova: Termiti, Isoptera, Blattodea: Termitoidae, hlavové exokrinní žlázy, rostrální žláza, mikroskopie

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Introduction

Termites (Dictyoptera: Isoptera; Blattodea: Termitoidae according to Lo *et al.* 2007, Eggleton *et al.* 2007) are one of the most ecologically and economically important insect groups on the world. Small-to medium-sized, and ranging from 3 to 20 mm in body length, they are familiar to common man due to their abundance, social organisation, caste system and division of labour, super architectural ability, a cryptic way of life, dependency on symbiotic microbiota and their pest status (Krishna *et al*. 2013). They are fully social insects, with a wide range of morphological forms. It is established that they are a very specialized form of cockroach, with far more complex social systems than other cockroaches, and with broader range of diets. All termites live in colonies.

Figure 1: *Soldier of* Coptotermes formosanus*, JŠ, pers. archive.*

The modifications seen in termite societies are comparable to the somatic parts of multicellular organisms, leading to the idea that a termite colony is best thought of as a single organism (Eggleton, 2011). Controversially, we think of it as a superorganism, where individuals are part of larger system and are dependent on each other (Wilson, 1992). The true social arrangement of insect societies (eusociality) is defined by three major traits: reproductive division of labour, mutual care for offspring and overlapping generation (Wilson, 1971). The division of termites according to their morphology and function into castes is following: reproductives (kings, queens, and neotenics), soldiers and “helpers” (true workers and immature stages that assist within the colony to some extent). Termite morphological and anatomical adaptations are caste-specific, with

structures evolving independently in reproductives (to allow dispersal, pair bonding and fecundity), workers (foraging and feeding, tending and feeding of immatures, nest construction) and soldiers (only defence) (Eggleton, 2010).

Termites can be found almost everywhere in the world from south to north. Epifamily Termitidae contains about 3000 described species (Krishna *et al.*, 2013). The level of diversity within the epifamily is modest, despite that they are among the most successful terrestrial organisms on the earth in terms of overall abundance. Termites are making up 10–20% of the animal biomass in tropical ecosystems worldwide and up to 95% of soil insect biomass (Eggleton *et al.*, 1996; Jones Eggleton, 2000; Tůma *et al.*, 2019). Termites (as well as ants and earthworms) are considered as soil engineers because of their effects on soil properties and their influence on the availability of resources for other organisms, including microorganisms and plants (Jouquet, 2006). They feed on dead plant material at various stages of decomposition. Their success is in large part due to their ability to digest lignocellulose and dissolved organic molecules in soil (Norkrans, 1963; Dixon *et al.*, 1994).

Aims of the thesis

1) Describe termite biology
2) Describe methodology and preparation of samples
3) Description of rostral gland in *Verrucositermes tuberosus* soldiers
4) Discuss my results and make a summary

1 Background

1.1. Ecology of Termites

Termites are abundant in tropical and subtropical parts of the world, maximum of diversity and abundance is concentrated around the equator (Eggleton, 2000). The trend with taxonomic diversity is for most of organism to decline as the temperatures decrease either from increasing elevation (Rahbek, 1995) or increasing latitude (Willig *et al.,* 2003). Termites show pattern of reduced diversity as elevation increases above ca. 800 m (Inoue *et al.,* 2006; Palin *et al.,* 2011) and as latitude becomes extra-tropical (Cancello *et al.,* 2014). But in some extreme cases termite species can also occupy environments in hot or arid deserts and in near freezing climates (Emerson, 1936).

Termites are decomposers. They digest plant tissues in all stages of decomposition, dead or decaying wood, grass, leaf litter, topsoil rich with humus or only mineralized organic compounds of soil (Bignell *et al.,* 2011). Through their diet, they influence soil topography, chemical properties, but also a plant growth rates (Jouquet *et al.,* 2006). However, plant tissues and their remains are very difficult to digest, so termites have developed intricate symbiotic associations with a number of microorganisms, bacteria, protozoa and fungi that help them to get the right nutrients out of their diet (Ohkuma & Brune, 2011). These symbionts allow termites to digest cellulose, hemicelluloses and lignin. Ability like this is not very common among animals and gave them huge advantage in evolution. Microbial symbionts also helped them to play a key role in processes such as carbon and nitrogen mineralization (Bignell & Eggleton 2000; Jouquet *et al.,* 2011). By their feeding habits and preferences for relatively undecayed living and dead plant material, about 10% of termites have been reported as pests. The caused damage can be to cultivated plants, timber, buildings, books, furniture, pastures and forests, but even to non-cellulosic materials such as electric cables (Bignell, 2000).

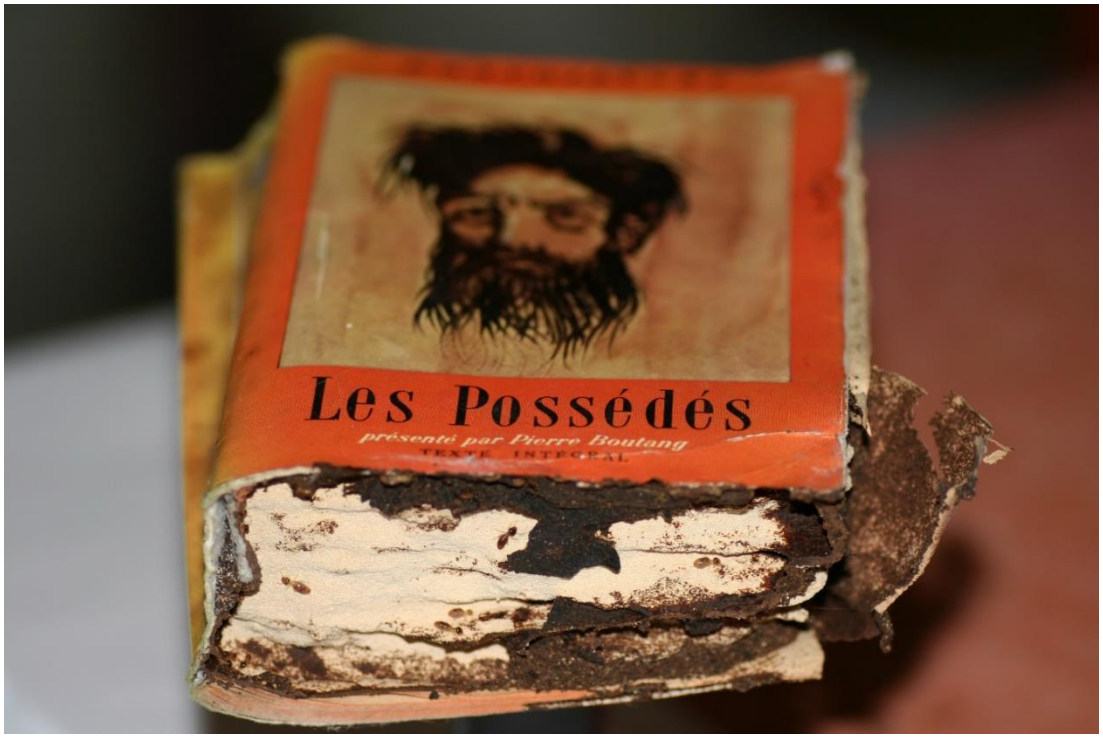

Figure 2: *Book partially eaten by* Nasutitermes, *JŠ, pers. archive.*

In the tropics, termites are arguably the most important soil ecosystem engineers (Bignell, 2006). Termites can forage over long distances (metres to tens of metres) and to partially control their own environments through the creation of nest structures where the humidity and temperature remain stable throughout all seasons. This gives them a striking ability to remain active in harsh environments, or during severe seasons, where most other soil macroinvertebrates are diminished or eliminated. For instance, in arid and semi-arid tropical savannas, during the dry season termites remain the only active group of invertebrate detritivores and bioturbators, consequently dominating the decomposition processes (Collins, 1983).

The cosmotropical family Termitidae, or "higher" termites, comprises approximately 70% of all termite species and appears to be the one of most ecologically significant adaptive radiations among insects. Monophyly of the Termitidae is well established; the family includes such familiar groups as the Macrotermitinae and Nasutitermitinae, some of which build huge mounds in grassland and scrub biomes; other nasute taxa build large arboreal nests from carton-like, faecal material in tropical forests (Engel *et al.*, 2009).

1.2. Phylogeny and classification of termites

Termites evolved from wood-feeding cockroaches and form a sister group with the cockroach genus *Cryptocercus* (Lo *et al*., 2000; Inward *et al*., 2007; Bourguignon *et al*., 2015). There is general agreement among systematists that cockroaches, mantids, and termites are phylogenetically closely related. The evolution of termites is of great interest, due to their diversity of diet, social structures, and phenotypes. A clear picture of termite ancestry is crucial for understanding how these insects evolved eusociality, particularly because they lack the haplodiploid genetic system associated with eusocial evolution in bees, ants, wasps and thrips (Lo *et al*., 2000). Termites split from *Cryptocercus* ancestor roughly 170 Ma, and Termitidae appeared roughly 54 Ma (Bourguignon *et al*., 2015). The first undisputed termite fossils are from the early Cretaceous, 110–135 Ma, and all belong to Mastotermitidae, Hodotermitidae, Kalotermitidae or to extinct families with unclear affinities to recent taxa (Krishna *et al*., 2013). Because termites are weak flyers (Hu *et al*., 2007), they are expected to be poor dispersers and the origin of their global distribution is enigmatic. While vicariance played a critical role in the early diversification of termites (Bourguignon *et al.,* 2015), the question is how termites dispersed across oceans.

Phylogeny of termites moved forward dramatically in the past 20 years. The reason for this fast development is the rapid development of molecular techniques. In the last 20 years, two major developments have transformed phylogenetic systematics: the extensive use of DNA sequence data and advances in methods of analyses. It is now accepted that DNA sequences are a wide source of characters for estimating phylogenies. The ability to obtain DNA sequence data quickly and cheaply has been achieved by the polymerase chain reaction (PCR) and the PCR-based cycle sequencing. As a result, we have short stretches of DNA, known as primers, that are used to amplify the target DNA and are available for a wide variety of genes and organisms (Kambhampati & Eggleton, 2010; Eggleton *et al*., 1996; Lo *et al*., 2000; Austin *et al*., 2004; Bourguignon *et al*., 2015).

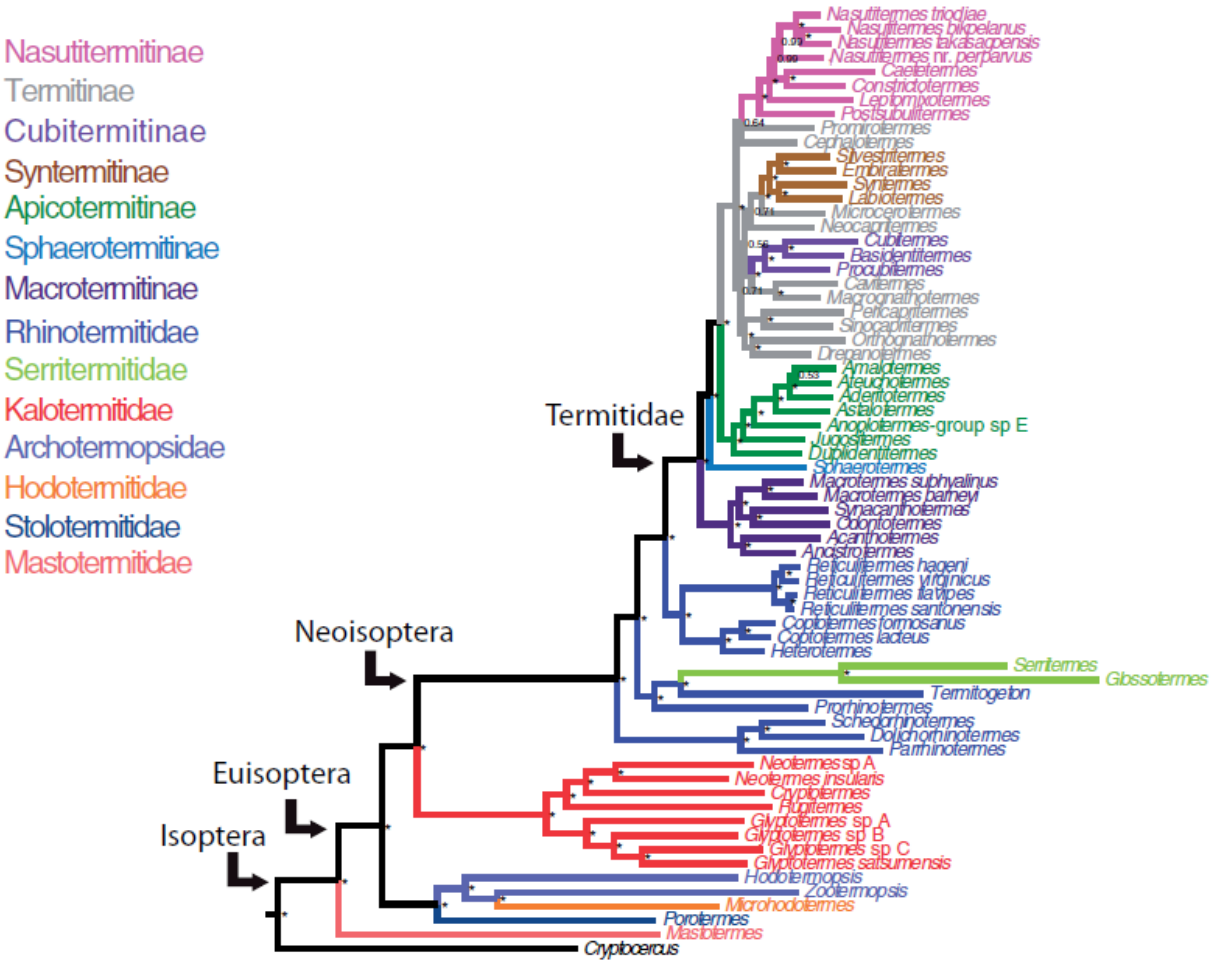

Figure 3: *Phylogenetic tree of termites: (a) Tree based on full mitochondrial genomes, reconstructed using Bayesian method and 20,000,000 generations. An optimal partitioning scheme was selected with PartitionFinder and third codon position was excluded from the analysis. Branch labels are the Bayesian posterior probabilities, with stars representing 100% support. Adopted from Bourguignon et al. (2015).*

On Figure 3, you can see a phylogenetic tree of termites by Bourguignon *et al*. (2015). This latest study on generic relationships between termites families was partially executed at Czech University of Life Sciences in Prague. On the bottom of the tree is *Cryptocercus*, sister group to all termites. Within termites, there are 2 large groups: Euisoptera and Neoisoptera. The basal group Mastotermitidae is a sister group to Euisoptera. Archotermopsidae, Hodotermitidae, Stolotermitidae form a monophyletic group, with Hodotermitidae based within a paraphyletic Archotermopsidae. Kalotermitidae were monophyletic and the sister group of Neoisoptera, which contains Stylotermitidae, Serritermitidae, Rhinotermitidae and Termitidae (Wu *et al*., 2018). Rhinotermitidae formed a polyphyletic assemblage with Serritermitidae recovered as the sister group of *Termitogeton*, which together form the sister group of *Prorhinotermes*. Termitidae is the sister group of *Reticulitermes*, *Coptotermes* and

Heterotermes. All subfamilies of Termitidae were monophyletic with exception of Termitinae which is polyphyletic. Macrotermitinae was the sister group of other Termitidae, followed by the monospecific Sphaerotermitinae. Among the remaining five Termitidae subfamilies included in this study, Apicotermitinae was placed as sister group of following four: Termitinae, Nasutitermitinae, Syntermitinae, Cubitermitinae (Bourguignon *et al*., 2015). The phylogenetic tree can be dived into "higher" (Termitidae) and "lower" (the eight basal families) termites. This division is however not taxonomically valid, but these groups represent a useful collective categories.

1.2.1. Mastotermitidae

This group has been accepted as the most phylogenetically basal extant species (Watson, 1991). It have probably evolved roughly 150 Ma (Bourguignon *et al*., 2015). In history fossil species of Mastotermitidae were present on all continents. Today, there is only one extant member *Mastotermes darwiniensis*, which is found only in northern Australia. Because *Mastotermes* is a basal group of Termites (Dictyoptera: Isoptera; Blattodea: Termitoidae according to Lo *et al.* 2007, Eggleton *et al.* 2007), therefore it was the first one to detached from roaches, they retain many plesiomorphies, such as: the anal lobe of hind wing, (internally placed) ovipositor, eggs arranged into ootheca or specialized fat body cells hosting symbiotic *Blattabacterium* symbiotic bacteria (Noirot, 1969; Ampion & Quennedey, 1981; Watson & Gay 1991; Grimaldi & Engel, 2008). At the same time, they have numerous advanced traits, such as: bifurcated ontogenetic patterns or highly potent defensive secretion acting as toxic and irritating agent and alarm pheromone, and sperm cells that reveal approximately 100 flagella (Moore, 1968; Gay & Calaby 1970; Baceti & Dallai, 1978; Noirot & Pasteels, 1987; Delattre *et al.*, 2015). *Mastotermes* feeds on wood and can be disastrous to buildings and other wood constructions. The underground colonies are of moderate size in natural habitats, but can be up to hundreds of thousands of individuals in anthropogenic habitats (Krishna *et al.*, 2013).

1.2.2. Archotermopsidae

Archotermopsidae *alias* damp-wood termites are paraphyletic group with respect to Hodotermitidae (Bourguignon *et al.*, 2015). The group contains three genera and seven species. Their distribution is wide from North America, through middle east to China. Their body size is large and colonies are small. Their ontogeny is linear without true

workers. Archotermopsidae feed on dead moist wood (Roisin, 2000; Krishna *et al.*, 2013).

1.2.2. Hodotermitidae

Hodotermitidae group contains three genera and 21 species. They are specialized grass-feeders and may be found in arid grasslands of Africa, middle East and south Asia. The family was distributed worldwide in the Cretaceous and Tertiary (Krishna *et al.*, 2013). Their nest are quite fragile and built underground. All species in this family forage outside and therefore they have developed eyes. They bodies are rather large, and they have been known to possess a true worker caste (bifurcated ontogeny) (Bignell *et al*., 2011).

1.2.3. Kalotermitidae

Kalotermitidae group contains 21 genera and 456 species. It is the second richest termite family (Krishna *et al.*, 2013). Kalotermitidae is a family of cosmopolitan distribution with highest abundance on tropical islands, in higher latitude or altitude locations. They are characterized as a good colonizers but weak competitors. They often colonize dead branches on living trees or hardwood in tropical lowland forests. They are able to feed on very dry hard wood, their mandibles are strongly reinforced with zinc (Bignell *et al*., 2011). Their body size is medium to large. Their colonies are of smaller size, usually not more than few hundreds of members. Their ontogeny is linear (Eggleton, 2000; Roisin, 2000).

Figure 4: Cryptotermes havilandi *soldier, JŠ, pers. archive.*

1.2.4. Stylotermitidae

Stylotermitidae is monophyletic group, which contains 1 genus and 45 described species. Representatives of this group are found in south-east Asia only. Stylotermitidae is the least known group of all, probably due to their feeding habits feeding on the freshly dead wood inside living trees (Krishna *et al.*, 2013).

1.2.5. Serritermitidae

Family Serritermitidae contains 2 genera and 3 described species. Termites of this group can be found only in the Neotropics (Krishna *et al.*, 2013). Both genera show a unique ontogeny, combination of linear ontogeny with sexual specialization. Workers and soldiers are always males (Bourguignon *et al.*, 2009; Barbosa & Constantino, 2017).

Figure 5: Glossotermes oculatus *soldiers, JŠ, pers. archive.*

1.2.6. Rhinotermitidae

Family Rhinotermitidae contains 12 genera and 315 species. The family is cosmopolitan in distribution (Krishna *et al.*, 2013). Rhinotermitidae is polyphyletic taxon, which can be divided into three monophyletic units: (*i*) Rhinotermitinae; (*ii*) Psammotermitidae containing *Prorhinotermes*, *Psammotermes*, *Termitogeton*, and Serritermitidae; (*iii*) Heterotermitidae, including *Reticulitermes*, *Heterotermes* and *Coptotermes* (Krishna *et al.*, 2013; Bourguignon *et al.*, 2015). Rhinotermitinae and

Heterotermitidae have a bifurcated ontogeny, whereas Psammotermitidae have a linear one (Roisin, 2000; Barbosa & Constantino, 2017). All soldiers have developed mandibules, modification in labrum may occur in Rhinotermitinae (Weesner, 1969; Prestwich, 1984). The frontal gland usually contains contact poison (Preswitch, 1984).

1.2.7. Termitidae

Family Termitidae contains approximately 70 % of species of termites and it is additionally subdivided into eight subfamilies (Krishna *et al.*, 2013; Bourguignon *et al.*, 2015), i.e. Macrotermitinae, Sphaerotermitinae, Foraminitermitinae, Apicotermitinae, Syntermitinae, Cubitermitinae, Termitinae, and Nasutitermitinae. They are the most ecologically dominant taxon especially in rainforests. Whole Termitidae group is absent of flagellates (Bignell *et al.*, 2011). Termitidae evolved in the late Eocene (~54 Ma), postdating the breakup of Pangaea and Gondwana when most continents became separated, and their recent distribution is a result of repeated colonization and vicariance events (Bourguignon *et al.*, 2017).

Figure 6: Aparatermes cingulatus *queen, JŠ, pers. archive.*

Ontogeny of Termitidae is always bifurcated and very uniform. Workers differ from larvae and nymphs. The caste system and sexual polymorphism reach the highest levels here (Noirot and Pasteels 1987; Roisin, 2000). Queen ovarioles are well developed and numerous, resulting into extraordinarily distended (physogastric) abdomen and high fecundity (Bignell *et al.*, 2011).

1.3. Life cycle

The life cycle is similar for all termites families. Mature colonies are periodically producing winged reproductives ("alates"). Most tropical species fly in the first part of the rainy season, but the actual date differs between species, determined by e.g. the cumulative amount of rain received. Alates usually leave the nest at once in swarm. Swarming is a collective behaviour exhibited by animals, of similar size which aggregate together and migrate in same direction. Swarming is in termites interpreted as an anti-predator herd-like defence. The time of swarming in termites may be affected by predation pressure. Many species swarm in dark, when predation by birds is at a minimum (Eggleton, 2011; Lepage & Darlington, 2000). These reproductives land on the ground drop their wings, female attract the male by a powerful sex pheromone and pair up. The pair dugs a chamber, future royal chamber, they mate, and the eggs are starting to be produced. Sometimes, the ground is only soft enough to dig into during the rainy season, significant share of newly-established colonies probably dies out before the next rain comes. The first cohort of offspring contains, apart of variable numbers of workers, a single soldier. The youngsters gradually overtake the essential functions, tend young, build colony structures, forage for food, and defend the hive and foraging area. The exponential growth of the colony eventually starts afterwards, and the colony maturity is recognized as production of the first cohort of alates allowing the cycle to repeat. It is important to realize how risky the dispersal flight is: *Macrotermes* (Termitidae: Macrotermitinae) colony may keep producing up to hundred thousands of imagoes a year for several decades and give rise on average to a single mature colony in whole existence upon condition that the overall population of a species remains stable (Grassé, 1984; Shellman-Reeve, 1997; Eggleton, 2011).

An alternative mechanism for colony reproduction is budding and production of neotenic reproductives. Budging is usually more successful than the foundation of new colony. Budding is a great way for termites to exploit new source of food or new habitat after long range dispersal. Long range dispersal by alates produces monogynous societies, while budding produces polygynous societies. Therefore, termites who also reproduce by budding may have a greater genetic variability. When several reproductives occur in a nest, genetic diversity could be an advantage in

adapting the species to new conditions (Grassé & Noirot, 1951; Grassé, 1986; Noirot, 1986; Lepage & Darlington, 2000).

***Figure** 7:* Macrotermes carbonarius *is getting ready for swarming, JŠ, pers. archive.*

1.4. Eusociality and termite caste system

Eusociality is the highest level of organisation any society can get to. Some animal species, especially an insect, can display an advanced level of social organization. Eusociality is characterized by cooperative brood care, overlapping generations within a colony, and a division of labour. The division of labour creates specialized behavioural groups, which are called castes. Eusociality is distinguished from other social systems because individuals of at least one caste usually lose the ability to perform at least one behaviour characteristic of individuals in another caste. Termite societies display a wide array of polymorphism. It can be matched only by the most complex ant societies (Bignell *et al.*, 2011).

A termite colony is a family of individuals living together. Generally, we can divide the colony into two parts an inanimate and an animate part. The animate part is the individuals living within the colony. The inanimate part is the structures – the nest built by individuals within which they live. The inanimate part of the colony can be just a few tunnels, but often it is a very extensive and sophisticated structure. Termite species can be separated into three categories: one-piece nesting - termites nest and feed on a single piece of wood, intermediate-type nesting - termites build subterranean

galleries to new pieces of wood, and separate-piece nesting - termites always forage or feed away from the nest (Abe, 1987). The animate part of a colony has, apart of immatures, brachypterous nymphs and short-term stages, typically three main castes: reproductives (queens, kings, and alates), workers, and soldiers. The queen is typically the only egg-laying individual in the colony, king is her consort and his only task is to mate with her on regular basis (Korb, 2008). The alates are winged reproductives preparing to leave the nest in order to swarm, to pair and to start new colonies (via chapter 1.3. Life cycle). Soldiers are the defenders of the colony and workers work as a providers, carers and builders. The main choice for every colony is to divide energy into maximizing the genetic gains but not to compromise the survival of the colony, to reproduce but at the same time to sustain nest homeostasis and to allow future reproductive success in the next year. The number of individuals in castes changes during the colony lifetime, most often varying seasonally and with sexual brood production. This would be a simple description of caste structure and dynamics as an ideal one, but things are unfortunately little more complicated (Lepage & Darlington, 2000; Eggleton, 2011).

Figure 8: Acanthotermes acanthothorax *queen, JŠ, pers. archive.*

Figure 9: Nasutitermes triodiae *nest, JŠ, pers. archive.*

1.4.1. Alates, queen and king

Alates' task in colony is to reproduce and go out to disperse. Alates are within termite colonies in their abilities closest to the solitary insects. In general they look like long, thin cockroaches. Alates interact with the outside environment much more than the other castes and we can use them to set out the basic body plan of termites because they look more similar to other closely related insect groups. Alate is an imago still possessing its wings. Dealate is an alate after shedding wings and becoming primary reproductives - king or queen of the new founded colony (Roisin, 2000). During the life of the couple, both individuals change in body structure. After they

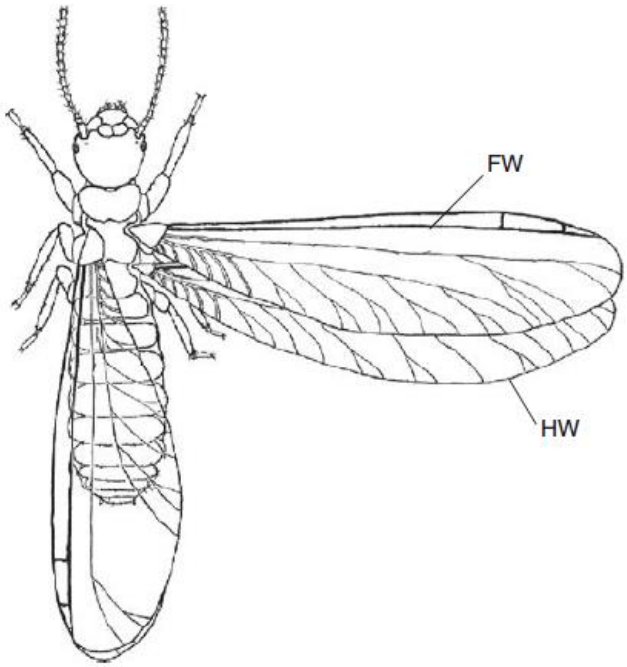

Figure 10: *Alate structure (illustrated by* Tenuirostritermes*), FW = forewing, HW = hindwing. Adopted from Weesner (1970)*

settle down the flight muscles degenerate and the queen's abdominal part enlarges multiple times (so-called physogastry). The couple remains inside the original chamber for the rest of their lives, and their needs are met by a working caste (Krishna, 1969). Like most insects a termite alate is split into three body regions – a head, a thorax and an abdomen. Each individual is equipped with slender wings in which generally look similarly (Fig. 10). Termite alates are generally poor flyers: their technique is to launch themselves into the air and fly in a random direction. All alates have eyes and one pair of lateral ocelli on the dorsal (top) surface: eyes are obviously necessary for dispersal and mate recognition. There is, however, considerable variation in key structures across the termite families (Eggleton, 2011)

1.4.2. The soldiers

The whole Isoptera clade is eusocial because of the possession of a soldier caste. There is little doubt about the evolutionary origin, development and function of soldiers. Soldiers evolved once in history of termites. All termite's families have a soldier caste except some derived soil-dwelling termitids, who lost them secondarily. It is important that this was secondary lost, not a primary deflect from ancestral lineage. Soldiers are a terminal and sterile caste. They usually develop from apterous instars, mostly workers, through an intermediate, unsclerotised instar, the presoldier (Roisin, 2011).

Soldier caste can take upon various roles. It is mainly to defend the colony, and particularly the queen and the king against predators and/or inter- and intraspecific competitors. Another role which this caste take is exploration, recruitment or even egg care (Haverty and Howard 1981; Roisin, 2011). Termite soldiers can be males or females, but their genitals are mostly stunted (except for fertile soldiers in the family Archotermopsidae). Soldiers are usually easily identified. Many of them reveal one to several types, the soldier subcastes. Soldiers show the greatest of variation of any caste, not only between species but also within. At the generic and species level soldier morphology is the most important source of taxonomic characters. These variations can be seen almost entirely in the head capsules of soldiers. Their head is enlarged and sclerotized compared to the worker. Modifications is also observed in labra, which can be transformed into various shapes. The significant changes are defensive adaptation such as modified and enlarged mandibles, which are used exclusively to defend against enemies (especially ants) (Weesner, 1970), or a frontal gland able to produce a copious amounts of defensive secretion. There are many variations of the mandibles - large,

small, long, short, symmetrical or asymmetrical (see Fig. 11 - a, b, c, d, e, f, g, h, i, k, l, m). Also the outlet of the frontal gland can be modified. The head can be extended into snout, where the frontal gland outlet is located at the tip (see Fig. 11 - j, n, p, o). Soldiers may have stunted eyes, but in most cases, they are completely absent. Due to the adaptation of mandibles for defense, individuals are not able to obtain food for themselves and are dependent on the colony (Roisin, 2000).

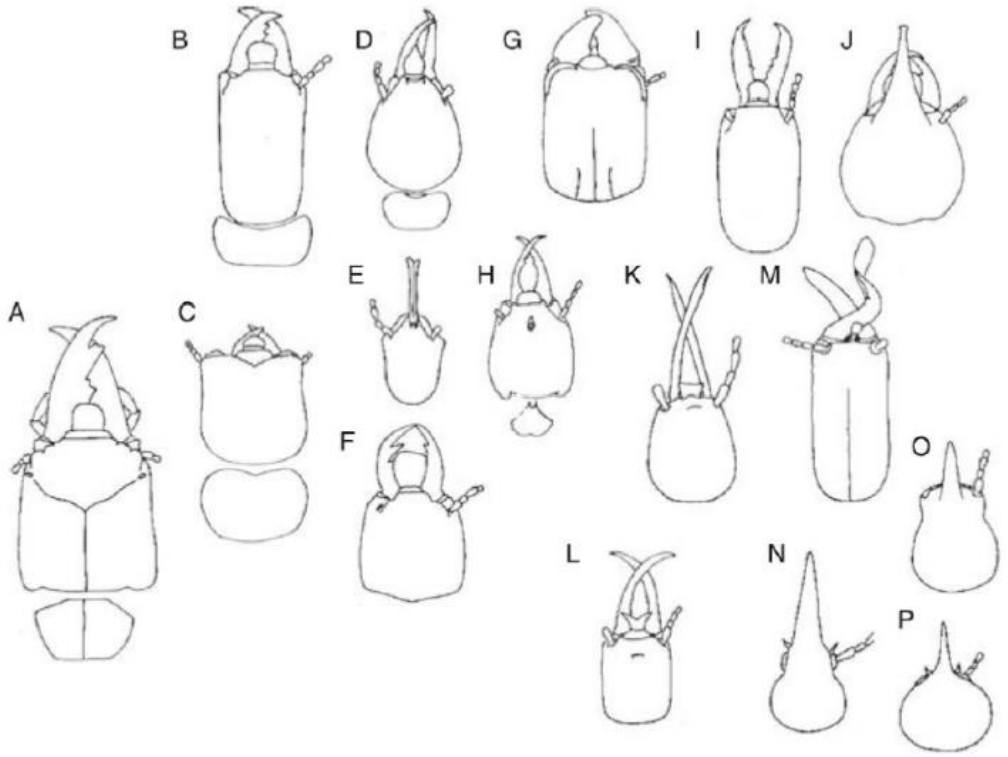

Figure 11: *Top view of soldiers' heads adaptations, edited from Weesner (1970).* A- Archotermopsis wroughtoni; B- Rugitermes bicolor; C- Cryptotermes verruculosus; D- Coptotermes sjostedti; E- Rhinotermes hispidus (small soldier); F- Rhinotermes hispidus (large soldier); G- Jugositermes tuberculatus; H- Acanthotermes acanthothorax (small soldier); I- Microcerotermes fuscotibialis; J- Armitermes grandidens; K- Promirotermes orthocopes; L- Procubitermes niapuensis; M- Pericapritermes urgens; N- Angularitermes nasutissimus; O- Coarctotermes suffuscus; P- Nasutitermes octopilis.

1.4.3. The workers

Workers have numerous roles. They forage for food and water, build and repair colony structures, feed the dependent castes, tend the immatures, alates, the king and the queen. Workers are usually of both sexes and are sterile, although in some groups they are able to differentiate into neotenic reproductives. This may happen when primary reproducers die, and workers must become replacement reproductive, or they can

differentiate into supplementary reproductives living along with the original ones. They spend most of their life underground. The worker's body is soft. The head, thorax and abdomen are essentially similar to those in the alates, except for the absence of wings and any genital structures. Almost all worker termites are blind, and they lack both compound eyes and oceli. The few exceptions are all early branching groups, some of which, but not all, forage above ground. However, there are also many blind surface foragers so the connection between above ground foraging and eyes may be mostly a phylogenetic signal. They move in the environment mainly with the help of communication pheromones and use high sensitivity to soil shocks (Eggleton, 2011). Workers have a well-developed pair of mandibles, a maxillae and a labium. Termite worker-imago mandibles are very variable in the number of their marginal teeth, but functionally they seem to fit into two groups: (a) grinding (milling), and (b) pounding (pestle and mortar) (Donovan *et al.*, 2001). In the oral cavity, a paired labial glands open, which secrete saliva and digestive enzymes that aid digestion, as well as a source of cement for the construction of termites (Noirot 1969).

Figure 12: Odontotermes *comb worker detail, JŠ, pers. archive.*

1.5. Ontogeny

The life cycle of termites is series of moults based on classical hemimetabolous metamorphosis. It has four basic stages: an egg, a larva, a nymph (brachypterous stage) and an adult. We distinguish two different patterns of ontogeny within termites: linear (A) and branched (B) (Fig. 13). Both pathways begin with the laying of eggs, which are (with minor exceptions) fertilized by the king's sperm.

The linear pathway is typical for lower termites and close to cockroach's ontogeny. In the linear type (A), the first larval instar is followed by several other instars. The individual is getting bigger which each moult which leads to the caste of a false worker, the pseudergate (Roisin and Korb, 2011). The term pseudergate is used for all older larvae that work. The number of apterous instars is unstable. If individual later develops foundations of wings we refer to it as "nymph". Nymphs still have the opportunity to develop into different form because they may lose their wings buds regressively (Roisin & Korb, 2011). Social responsibilities are shared equally by both sexes (Roisin, 2000). Species with linear postembryotic development belong to the families Archotermopsidae, Stolotermitidae, Kalotermitidae, Serritermitidae and the genera *Prorhinotermes, Termitogeton* and *Psammotermes* (Rhinotemitidae) (Roisin & Korb, 2011).

In branched ontogeny, there is an early division into two branches, apterous line and nymphal-alate line. The apterous line gives rise to all workers (true workers) and soldiers. The division is irreversible and separates sterile and fertile castes from each other during development. This scheme is typical for the families with large colonies with up to million individuals such as Mastotermitidae, Hodotermitidae, Termitidae and for some genera of Rhinotermitidae (Roisin & Korb, 2011).

Linear pathway is connected with instable environments, smaller colony size (under thousands of individuals), lower polymorphism and lower sex differentiation compared to bifurcated ontogeny (Roisin, 2000). The development of an individual is not predetermined. It is regulated by pheromones produced by members of individual castes. On the other hand, evidence on the influence of genetic factors on an individual's future has recently begun to emerge. The rate of development is

also variable and is influenced by a number of factors, such as the availability of food or the number of individuals in each caste (Krishna 1969).

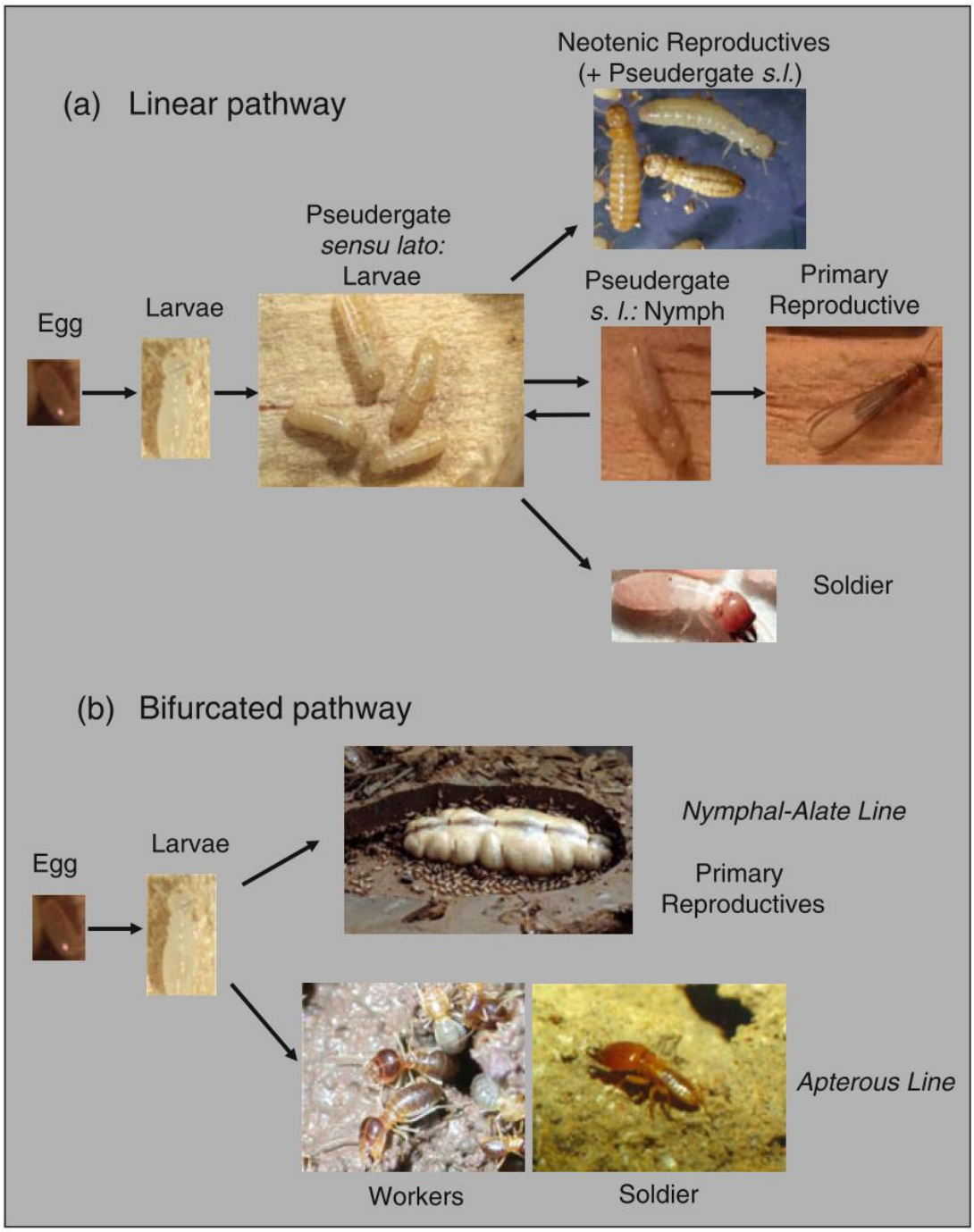

Figure 13: *Simplified developmental pathways of (**a**) wood-dwelling termites with a linear development and (**b**) foraging termites with a bifurcated development. In wood-dwellers, pseudergates sensu lato are totipotent to develop into primary reproductives, neotenic reproductives and soldiers. In foraging species, the developmental lines separate early on into a nymphal and an apterous line, the former leading to winged sexuals and the latter to workers and soldiers. In many species workers still have the potential to become reproductives. Adopted from Roisin & Korb (2011).*

2 Termite glands

Termites have developed many exocrine glands. The termite set of exocrine organs is rich compared to that of solitary insects. The use of exocrine glands by termites is undoubtedly connected with the need of communication in eusocial insect communities and protection of the nest. A wide variety of such glands has been described, their major function often being the production of pheromones, the source of digestive enzymes and defence (Billen, 1989). 23 glands have been described in termites so far. Each termite species contains at least 5 different glands. Some of following glands can occur in all termite species, or represent synapomorphies of larger clades, others are morphological innovations (Šobotník, 2015).

Most termite species possess these following glands*:* the frontal gland, the sternal gland, the labial glands, the mandibular glands, the labral gland, and tergal glands (Fig. 14) (Šobotník, 2015). The occurrence of another exocrine glands depends on termites species and its caste (Palma-Onetto *et al*., 2018). As there are glands which are common for most termites, there are of course glands which are specific for only certain termite lineages. For example mandibular base glands of soldiers of *Machadotermes* (Termitidae: Apicotermitinae; Quennedey, 1984), nasus gland of soldiers of *Angularitermes* (Termitidae: Nasutitermitinae; Šobotník *et al.*, 2015), rostral gland of soldiers of *Verrucositermes* (Termitidae: Nasutitermitinae; Deligne, 1983), crystal gland of *Neocapritermes taracua* workers (Termitidae: Termitinae; Šobotník *et al.*, 2014) or dehiscent glands in *Ruptitermes* workers (Termitidae: Apicotermitinae; Costa-Leonardo, 2004; Poiani & Costa-Leonardo, 2016).

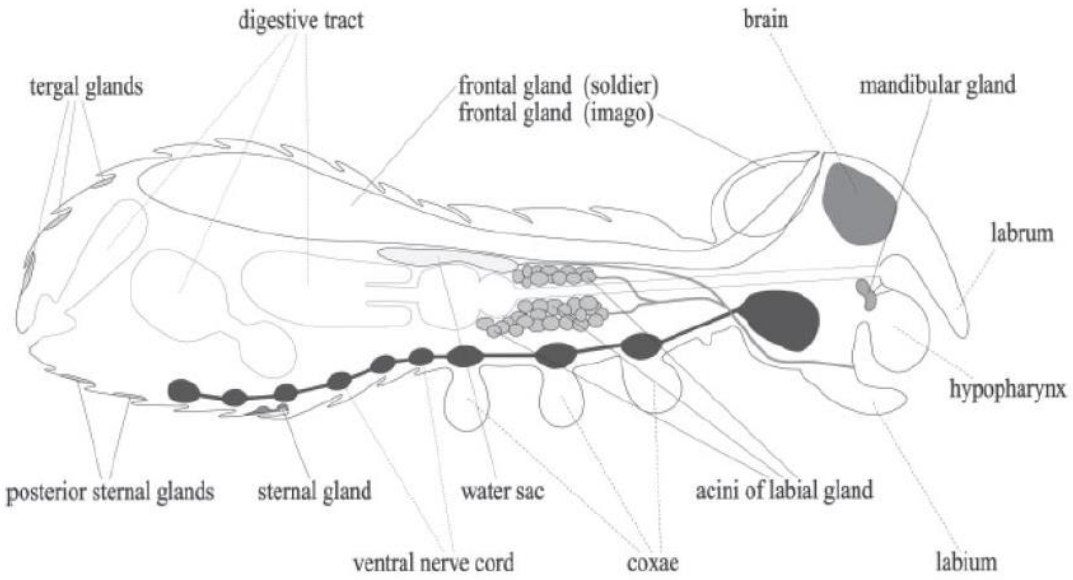

Figure 14: *Termites basic morphology. Location of the most common glands. Modified from Šobotník & Hubert (2003)*

I have divided the described glands into 4 groups due to their location in termites body:

Cephalic glands: frontal gland (Quennedey, 1984), labral gland (Deligne *et al*., 1981; Quennedey, 1984), clypeal gland (Křížková *et al*., 2014), hypopharyngeal gland (Brossut, 1973), mandibular glands (Lambinet, 1959; Noirot, 1969), accessory mandibular glands (Greenberg & Plavcan, 1986), nasus gland (Šobotník *et al*., 2015), rostral gland (Deligne, 1983), labial glands (Noirot, 1969; Billen *et al*., 1989), oral gland (Synek *et al*., 2019), intramandibular glands (Deligne *et al*. 1981), mandibular base glands (Quennedey, 1984).

Thoracic: tarsal glands (Bacchus, 1979), lateral thoracic glands (Gonçalves *et al*., 2010), dehiscent glands (Costa-Leonardo, 2004; Poiani & Costa-Leonardo, 2016), crystal glands (Šobotník *et al*., 2012; 2014).

Abdominal: sternal gland (Ampion & Quennedey, 1981; Quennedey *et al.*, 2008), posterior sternal glands (Quennedey *et al.*, 2004; Šobotník *et al.*, 2005), pleural abdominal glands (Ampion, 1980), tergal glands (Ampion & Quennedey, 1981), posterior tergal glands (Costa-Leonardo & Haifig, 2010), spermathecal gland (Raina *et al.*, 2007)

Whole body glands: epidermal tegumental glands (Šobotník *et al.*, 2003), integumental glands (Sbrenna & Leis, 1983).

2.1. Terminology and morphological classification

Gland: any glandular structure, from an isolated gland cell to a complex assemblage of glandular units, sometimes with associated differentiation for the storage, the emission or the evaporation of the secretion.

Gland cell: a cell which produces a secretion or a part of it.

Glandular unit: the unit must at the same time be structural, functional and ontogenetic.

- Structural: several cells are assembled in an organule, a set of cells which cannot be divided in two or more identical subsets.
- Functional: single secretion, even when more than one gland cell is present.
- Ontogenetic: all the cells of the unit are the offspring of one epidermal cell (stem or mother cell) as a result of one or more mitotic cycles. The unit is thus an isogenic group (clone) (Noirot & Quennedey, 1991).

Classification of glands is very difficult task. Insect glands are diverse in morphology, function, location etc. One gland can have multiple functions such as poison gland of the ants, principally defensive, is frequently utilized also for the deposition of a trail pheromone. Therefore, the following classification is focused on the gland cells (Noirot & Quennedey, 1991).

2.1.1. Class I gland cells

The class I cell is located right under cuticle and is in direct contact with it. The secretion is delivered by the cell itself. Cuticle can be modified to varying degree or sometimes, no cuticle is secreted in a part of the apical surface at all, producing a pore for secretion. Ontogeny of class I gland cells is simple. All cells are descendants of epidermal cell through mitosis (Noirot & Quennedey, 1991). For example, oral gland is formed by class I gland cells (Synek *et al.*, 2019).

2.1.2. Class II gland cells

Class II gland cells are always part of the epidermis. They can be either completely surrounded by epidermal cells or in contact with the epidermal basal lamina. They do not contact the cuticle and their secretion cannot reach the outside directly. It must pass either through the adjacent epidermal cells or through haemolymph (Noirot & Quennedey, 1991). Those cells are not very common, they have only been described

in a few glands for example in sternal glands of many termites (Quennedey *et al*., 2008).

2.1.3. Class III gland cells

Class III gland cells are connected with cuticle always via a cuticular canal. The canal is built from one or more other cells. Therefore, a class III gland cell is always integrated into a glandular unit. Such units may be isolated as dermal glands, or associated with other units (or the same or another type).

The secretory cell lies on the ental end of canal cell. It is typical for the secretory cell to be very active. It contains high number of mitochondria, endoplasmic reticulum, Golgi apparatus. Its nucleus is loose due to ongoing transcription and with well visible nucleolus. The cell has an invagination, the extracellular reservoir, lined with microvilli, in communication with the canal. The canal cell may either abut simply at the opening of the reservoir or extend into it with a loose type of cuticle, sponge-like or perforated. It is called receiving canal. We are using term end apparatus to name the whole system. The rest of the canal (up to the surface) is called conducting canal (Pasteels, 1968). The canal cell is typically inactive with condensed nucleus. It is quite usual for the gland unit to be made up of only two cells, terminal and canal cell around the conducting canal.

There are cases where unit can be also made up from three cells. The secretory cell and canal cell are joined with another cell. This cell is intercalated between these two, lying around the base of the conducting canal. Frequently this cell also contains a reservoir, crossed by the conducting canal, which is often dilated at this level, but the cuticle here is not as obviously porous as in the receiving canal. Nevertheless, the secretion of the intercalary cell must pass through this in order to mix with that of the terminal cell and be finally secreted (Noirot & Quennedey, 1991).

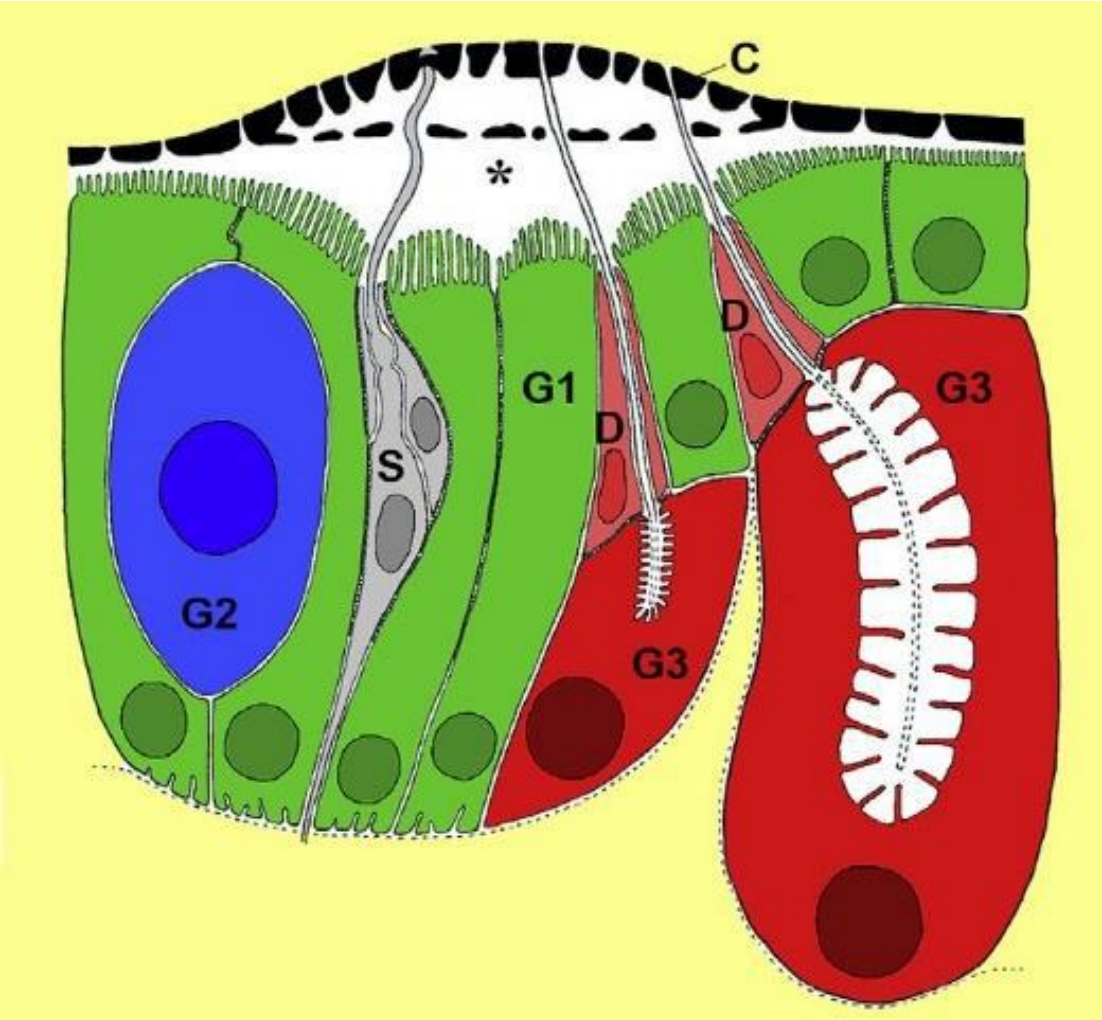

Figure 15: *Scheme of a sternal gland in "Rhinotermitidae" showing all 3 classes of secretory cells. Adopted from Billen & Šobotník (2015). * - subcuticular space, c – cuticle, D – duct cells, G1 – secretory cells class I, G2 – secretory cell class II, G3 – secretory cells class III.*

2.2. Frontal gland

The frontal gland is a structure without any equivalent among other animals (Noirot, 1969). It is the most prominent defensive organ of Neoisoptera - advanced termite families (Stylotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae). The gland can be reduced in some families such as Macrotermitinae and Termitinae. The frontal gland is present across the castes. It has been described in soldiers and reported in presoldiers, some workers and also in most imagoes (Šobotník *et al*., 2010c; Kutalová, 2013). The gland has been best examined in soldiers. It is unpaired, epithelial gland with reservoir (Billen, 2011). It is sac like organ which opens at the top of the head through the fontanelle. It can either be restricted only to the head, as in many Termitidae (Noirot, 1969), or reach deep into the abdomen as in most Rhinotermitidae and Serritermitidae (Šobotník *et al*., 2004). The frontal gland is in workers present as an epithelial thickening only (Šobotník *et al*., 2010b), while both forms were observed in imagoes (Šobotník *et al*., 2010c; Kutalová, 2013). The gland is usually formed by class I cells but there has been described formation of class I cells with class III cells (Quennedy, 1984; Šobotník, 2010c). The gland produces variety of chemicals with manifold purposes. The most obvious is defence and communication. The produced chemicals are classified as hydrocarbons, alcohols, aldehydes, ketons, macrolactones, mono-, sesqui- and diterpenes, aromatic compounds, nitro-compounds, ceramids and others. They can act as contact poisons, irritants, repellents, immobilizing agents, antihealants, or alarm pheromones (Prestwich, 1984; Šobotník *et al.*, 2010a; Roisin,1990).

2.3. Labial gland

Labial glands (also called salivary glands) are present in all castes and developmental stages of all termite species. Labial gland is a paired organ, which consists of numerous clumps of secretory cells (the acini), connected by a branching salivary canal (acinar duct). Each labial gland possesses a thin walled reservoir - water sac. The water sac emerges from the salivary canal (Noirot, 1969). The function of labial glands in termites is diverse. Labial glands are the most complex glands in termites since their products perform diverse roles such as: digestion (Noirot, 1969), communication (Kaib and Ziesmann, 1992) and defence (Deligne *et al.*, 1981; Prestwich, 1984). They were also observed to provide food for dependent castes, material for building activities (Noirot, 1969; Grassé, 1982) or mycostatic substances (Grassé, 1982). The

gland reservoirs are used for water retention. The water is used for regulation of microclimatic conditions in nests or moistening of material during building activities (Grube and Rudolph, 1999a).

Labial glands in *Prorhinotermes simplex* (Rhinotermitidae) consist of secretory cells organized into acini, water sacs and the ducts connecting the gland parts to the basis of the labium. Acini are composed of central and parietal cells. Central cells can be various types (I., II. or III.). The parietal cells are uniform. The water sac functions is probably a water storage only. The acinar ducts originate inside the acinus where they are formed by flat cells with rare organelles. Cells to be find on the acinus border are equipped with mitochondria, microvilli and basal invaginations. The water sac ducts are formed by flat cells (Šobotník & Weyda, 2003).

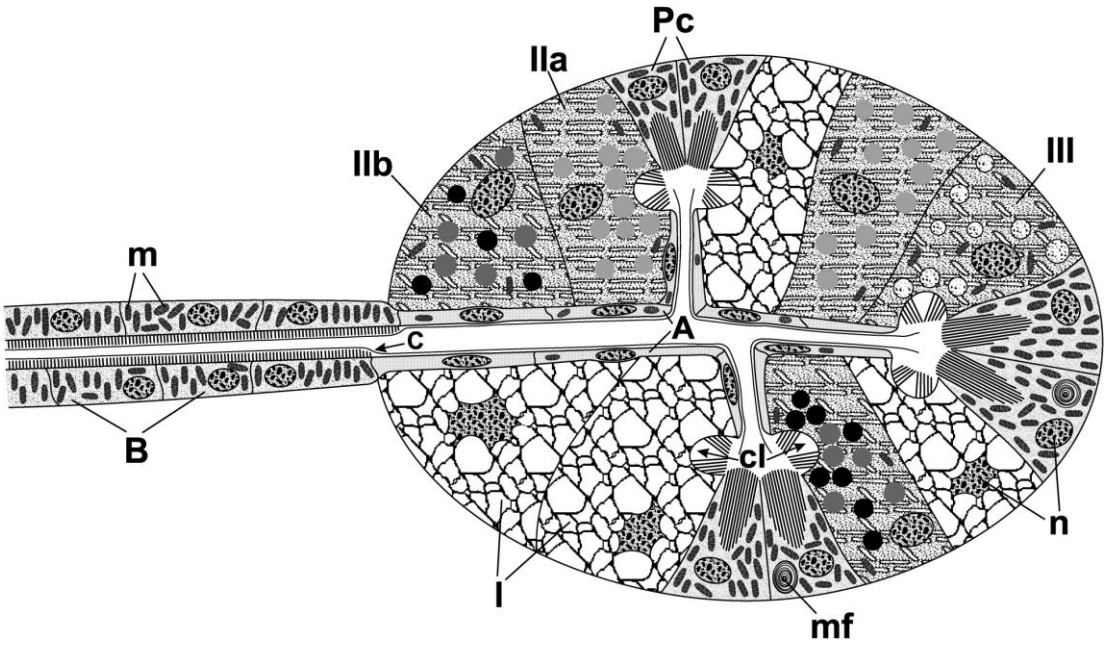

Figure 16*: Schematic drawing of an acinus in pseudergate. I, type I central cell; IIa, type IIa central cell; IIb, type IIb central cell; III, type III central cell; Pc, parietal cell; A, type A duct cell; B, type B duct cell;c, cuticle; cl, cell lumen; m, mitochondria; mf, myelin figure; n, nucleus. Adopted from Šobotník & Weyda, (2003).*

2.4. Labral gland

The labral gland is present in all species and belongs thus to the basic body plan of termites. It has been observed in soldiers, workers and imagoes of all species studied so far. It comprises two secretory regions located on the ventral side of the labrum and the dorso-apical part of the hypopharynx. The labral gland is an integral part of the labrum, which is a thin lip-like structure that covers the dorsal side of the pre-oral cavity (Palma-Onetto *et al.*, 2019). Common modifications are a higher degree of

sclerotization of the dorsal side of the labrum, occurrence of class III secretory cells at the dorsal side of labrum but rarely within the labral gland, and the presence of the labral gland formed class I secretory cells on the ventral side of labrum and on the dorsal side of hypopharynx. The secretory cells are similar in their ultrastructure, showing well-developed apical microvilli with a central channel, numerous vesicles of different electron densities, abundant smooth and rough endoplasmic reticulum, cuticle modified for secretion release, and innervation of the secretory cells through axons running freely within the basal invaginations (Palma- Onetto *et al*., 2018). The labral gland function is defensive communication (Palma- Onetto *et al*., 2019).

2.5. Sternal gland

Sternal glands are observed in all species and in all castes. As its names suggests the glands are located on abdominal sternite(s). It plays important role in the termite's societies, because it is responsible for secretion of the trail and the sex pheromones. The number of sternal glands and their positions within termites body can vary.

In the family Mastotermitidae, we find three sternal glands (on the third, fourth and fifth abdominal sternite) formed only by class I cells (Ampion and Quennedey, 1981). Families Archotermopsidae, Hodotermitidae and Stolotermitidae, have the gland on the fourth abdominal sternite. In the family Archotermopsidae tergal gland is formed by cells I. and II. class. Family Hodotermitidae has all three types of cells. The sternal gland is in the fifth abdominal sternite for all other families. In the family Kalotermitidae is the gland formed by cells I. and II. class. The most complex structure of the sternal gland occurs in the family Rhinotermitidae, where the gland is formed by cells I. and II. class and two cell types of class III. In the family Termitidae, the gland is always formed by cells I. and II. class. Subfamilies Macrotermitinae and Termitinae are exceptions and contain cells III. class as well (Ampion and Quennedey, 1981; Noirot, 1995). The secretion is stored in a separate reservoir formed by the front part of the 5th sternite covered by the back part of the 4th sternite. Groups of campaniform sensils are present in sternal glands in all termite families. Their function is to inform about the pressure of the abdomen on substrate and thus control the release of pheromones (Stuart, 1963).

The glandular cuticle is made of an outer epicuticle pierced by epicuticular pores, an inner epicuticle and a thick layer of epicuticular filaments. The size of the pierced

epicuticular pores is 30 - 50 nm in diameter allowing the excretion of larger molecules through the cuticle (Quennedey *et al.*, 2008).

2.6. Tergal gland

Tergal glands are usually located on last two or three abdominal tergites of alate imagoes and only in females in the advanced families Rhinotermitidae and Termitidae. Many taxa have them secondarily reduced and their function is then taken over by the sternal gland. Tergal glands are located on posterior tergites, most commonly on the last two or three. The tergal and the posterior sternal glands share the same structure (Noirot, 1969; Ampion and Quennedey, 1981). Tergal gland was found to be present in both sexes of *Mastotermes darwiniensis* (Mastotermitidae). In males they were located on sixth to ninth sternites, and on sixth and seventh in females. In genera *Porotermes*, *Stolotermes* (Stolotermitidae) and *Prorhinotermes simplex* (Rhinotermitidae) they have been described only in males, on eighth and ninth sternites (Ampion and Quennedey, 1981; Šobotník *et al.*, 2005). Tergal glands are composed of class I, II and III cells. The only identified compound produced by the tergal glands in termites is (3*Z*, 6*Z*, 8*E*)-dodeca-3,6,8-trien-1-ol, which has been described as a sex pheromone in *Cornitermes bequaerti* (Termitidae: Syntermitinae). The same substance is produced by the sternal gland in a number of termite species as a trail pheromone and may also act as a sexual pheromone but in higher concentrations (Noirot, 1969; Šobotník *et al*., 2005).

2.7. Mandibular gland

Mandibular glands are observed in all termite species, castes and all development stages (Holmgren, 1909; Noirot, 1969; Brossut, 1973; Cassier, 1977; Šobotník & Hubert, 2003). However, there is relatively little other information. The paired mandibular gland is formed by secretory class III cells located at the mandible base. It is connected with duct cell which leads secretion to oral cavity (Šobotník & Hubert, 2003). Size of the gland varies between species and also between castes within one species. Large mandibular glands have soldiers of the subfamily Nasutitermitinae (Termitidae), who's mandibles are completely stunted. Therefore, it has been speculated about the defensive function of these glands (Constantino and Costa-Leonardo, 1997). In *Kalotermes flavicollis* (Kalotermitidae), are the glands significantly larger in sexual individuals (Noirot, 1969). In *Prorhinotermes simplex* (Rhinotermitidae), the glands are largest in pseudergates and adults (Šobotník and

Hubert, 2003). Function of the mandibular glands is unknown and may vary between species. The size of the gland correlates with the presumed chewing activity of the different castes in the species *Prorhinotermes simplex*, so prevention of damage to the mandibular condyles can be assumed (Šobotník and Hubert, 2003).

3 Genus *Verrucositermes*

Genus *Verrucositermes* is a member of subfamily Nasutitermitinae (Termitidae) and belongs to informal *Subulitermes*-group. Main features of this group are apical and marginal mandibular denticles of workers and imagoes. Another adaptions are workers' molar blades and humivorus diet. *Verrucositermes* soldiers stand out from all the rest of soldiers of *Nasutitermes* by the presence of tuberucles on their head. There has been described two species *Verrucositermes hirtus* and *Verrucositermes tuberosus* so far, both living in Africa (first described in Gabon and Cameroon) (Deligne, 1983).

Soldiers have a thin nose approximately as long as the rest of the head. Their head is prominent with approximately 150 tuberucles. The tuberucles are particularly dense in the dorsal part of the forehead and base of the nose as well they are rare or absent on the ventral side and back of the head (Deligne, 1983)

***Figure 17**: Soldier of* Verrucositermes tuberosus, *JŠ, pers., archive*

Figure 18: *Soldier of* Verrucositermes tuberosus*, JŠ, pers., archive*

3.1. Rostral gland

The rostral gland has been observed in soldiers of *Verrucositermes hirtus* (Nasutitermitinae) (Deligne, 1983) and unrelated *Embiratermes festivellus* (Syntermitinae) (Costa-Leonardo & Barsotti, 1996). The ultrastructure of this gland has never been studied but the observation from a scanning electron microscope (Fig. 19) suggests that it is composed of cells III. classes (Deligne, 1983). The function of those secretory cells is unknown, it has been suggested that it can be production of secretions that prevent soldiers from getting stuck in their own sticky secretions (Deligne *et al.*, 1981).

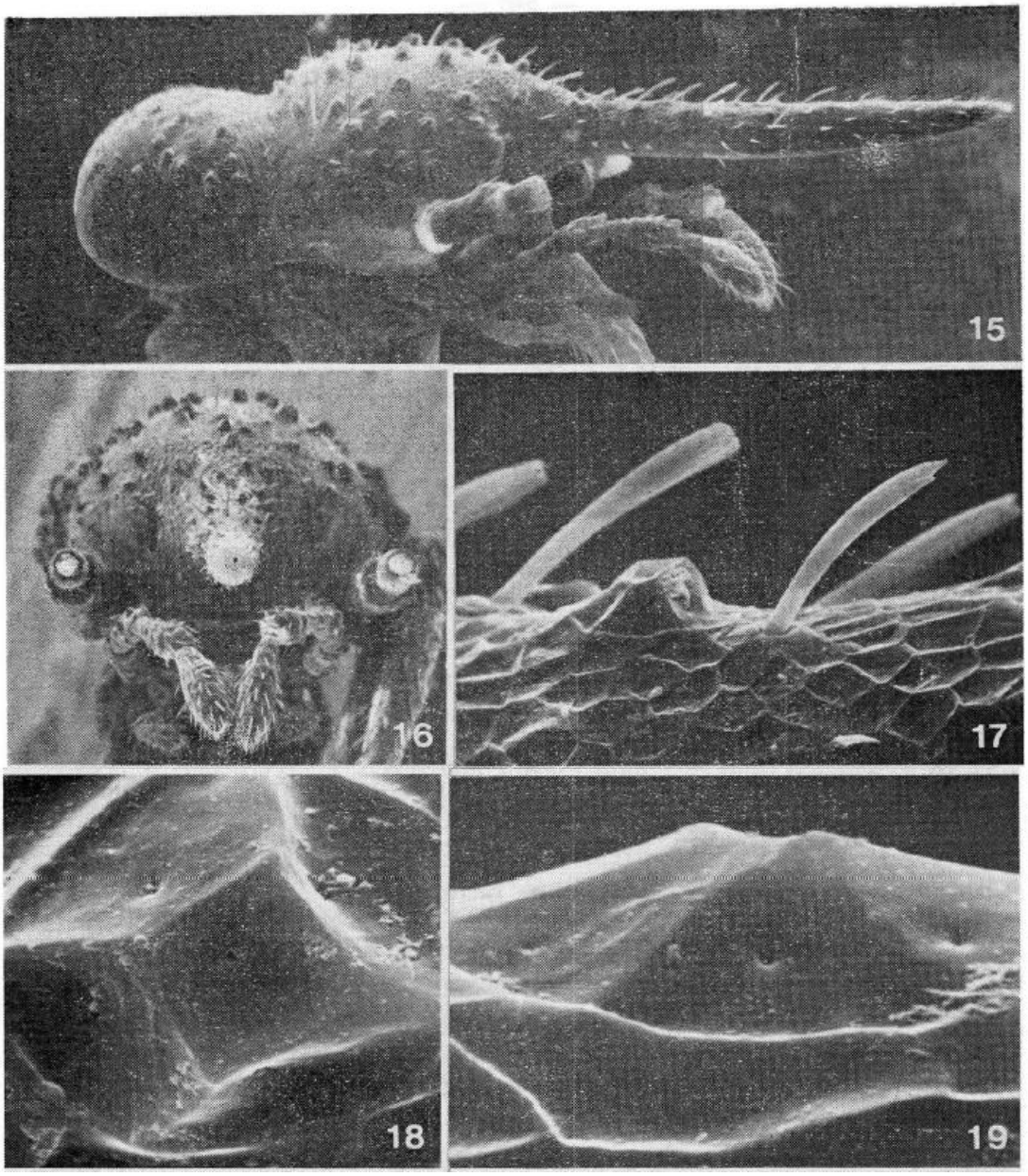

Figure 19: *No. 15. – 19., ultrastructure of head and tuberucles of soldier of* Verrucositermes hirtus*, viewed with scanning electron microscop.15. Head (without antennas) side view (x 90);16. Head from the front (x 100);17. Tuberucle and bristles at the base of rostrum (x 760); 18. Tuberucle from above with glandular pores (x 2000); 19. Profile of tubercule with visible glandular pores (x 4000). Modified from Deligne* et al.*, (1981).*

4 Methodology and sample preparation

The aim of the practical part of my thesis is to confirm the nature of the tubercles occurring on the head of soldiers of *Verrucositermes tuberosus.* These structures were described to be connected to yet-unknown exocrine organ called rostral gland based on scanning electron microscopy observation. To observe this exocrine organ, we used techniques of optical and transmission electron microscopy.

4.1. Preparation of samples

4.1.1. Fixation of samples

Primary fixation: The head of termite was placed in a drop of primary fixative consisting of 0.2 M PBS (phosphate buffer) with 7.2 pH, 8% glutaraldehyde and 10% formaldehyde at a ratio of 2: 1: 1. The fixation of sample took 3 days at + 4 ° C. Next step was rinsing with 0.1 M PBS (0.2 M PBS and distilled water in a ratio 1: 1). The rinsing was repeated 3 times.

Post fixation: It was performed with a 1:1 solution of 2% osmium oxide and 0.1 M PBS (phosphate buffer). The post fixation took 2 hours.

Washing with distilled water: After fixation, it was necessary to wash the samples with distilled water. The washing was performed three times in intervals of twenty minutes.

Dehydration: It was performed by gradually increasing the concentration of the acetone solution, to finish with pure 100% concentrate. The intervals for each concentration were: 30% acetone solution for twenty minutes, 50% for twenty minutes, 70% for thirty minutes, 80% for thirty minutes, 90% for twenty-five minutes, 95% for thirty-five minutes and finally the samples were left in 100% acetone for seventeen hours in the refrigerator.

Resin embedding: Embedding is done by gradually increasing the concentration of Spurr resin (standard mixture) in acetone. First, the samples are stored for one hour in a 1:2 solution of Spurr resin and acetone, then we adjust the concentration to 1:1 for one hour, and finally the Spurr concentration is increased 2:1 again for one hour. Finally, we leave the sample for twenty hours at 4 ° C in pure Spurr resin.

Polymerization: at +70°C for 8 hours.

Figure 20: *Fixed samples of* Verrucositermes *tuberosus, used samples 161/1,161/6 and 161/13 are marked, JŠ, pers. archive*

4.1.2. Microtomy and slide preparation

Sample sectioning: We used Reichert-Jung microtome and glass knives to section the resin blocks. Sliced samples were placed on glass slides that were precleaned with 80% ethanol and cotton swabs. To transfer the sliced samples from the knife we used a glass rod with a ball at the end. To dry the samples on slides we used a hot plate set at +60°C. Razor blades were used to trim the resin block into a desired shape. I used pipettes and a syringe with a needle to dispense 10% acetone solution. Methylene blue dye (1% solution in 1% borax) and pipettes were used for staining and distilled water was required for a rinse. For observation we used optical microscope Nikon Eclipse *Ei*.

Sample preparation: The specimen block had to be adjusted prior to sectioning on the microtome. We fixed the resin block in one of the microtome holders, which was inserted under the optics of the microtome, and trimmed the excess resin around the fixed sample (head of termite in our case) with a razor blade to create as small cutting

area as possibly around 2 mm^2. The desired shape of block was a trapezoid with our sample on the top. This procedure reduces the pressure of the specimen block on the knife, so that the samples will not crease. It also allows us to easier move the specimen along the blade so that the cutting edge was as sharp as possible. It is desirable to move the blade often and frequently replaced it. This prevents damages such scratches or squeezes on the cuts and thus their final deterioration.

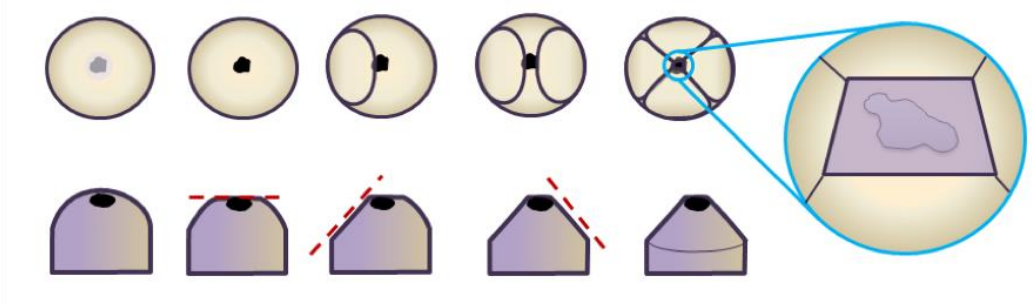

Figure 21: How to trim a resin block for microtomy. *A razor blade can be used to trim away the resin, first exposing the surface of the sample and then trimming away the edges until a trapezoid shape is produced. Modified from Hagler (2007).*

Microtomy: It is a process of cutting tissue into a thin section. In our case we used rotary microtome Reichert-Jung with glass knife. Thin section or "slices" prepared with this method are fitted for light microscopy. The prepared sample was attached to the head (holder) of the microtome pendulum. We attached a glass knife with a little plastic pool filled with 10% acetone solution. The samples were cut to thickness of 0.5 μm. After cutting, the slices slid from the blade of the knife into space with acetone solution. Low -quality slices were removed, and high-quality slices were transported with glass stick to glass slides. These slides were first degreased with ethanol and then we prepared drops of acetone solution. One drop of acetone contained approximately 20 or 30 semithin sections. After the semithin sectioning, the samples were numbered with a code consisting of the sample number (e.g. 161/11), and **a** or **b** for the different positions within the resin block.

The numbered slide was transferred to a hot plate heated to 60 ° C. Our prepared slices glued to the slide by drying. Subsequently, the individual sections were dripped with Methylene Blue dye and transferred back to the hotplate for about 30 seconds and washed with distilled water after. Prepared slides are ready for observing with a optical microscope.

4.1.2. Ultramicrotomy

Ultramicrotomy is process very similar to semithin sectioning except the prepared sample is usually used for imaging by transmission electron microscopy (TEM) or techniques using scanning electron microscopy. The sample is cut into extremely thin slices or "sections". The typical thickness of these sections is usually around 50 nm, the thinner the better, but however we are meeting here the physical constraints given by the resin properties. The ultramicrotomy uses diamond knives (preferably) or glass knives. To collect the sections, they are floated on top of a liquid as they are cut and are carefully picked up onto grids suitable for TEM specimen viewing or it can be picked up by eyelash brush and transported (Fig. 3). We have used a grid method. The thickness of the section can be estimated by the thin-film interference colours of reflected light that are seen as a result of the extremely low sample thickness (Hagler, 2007).

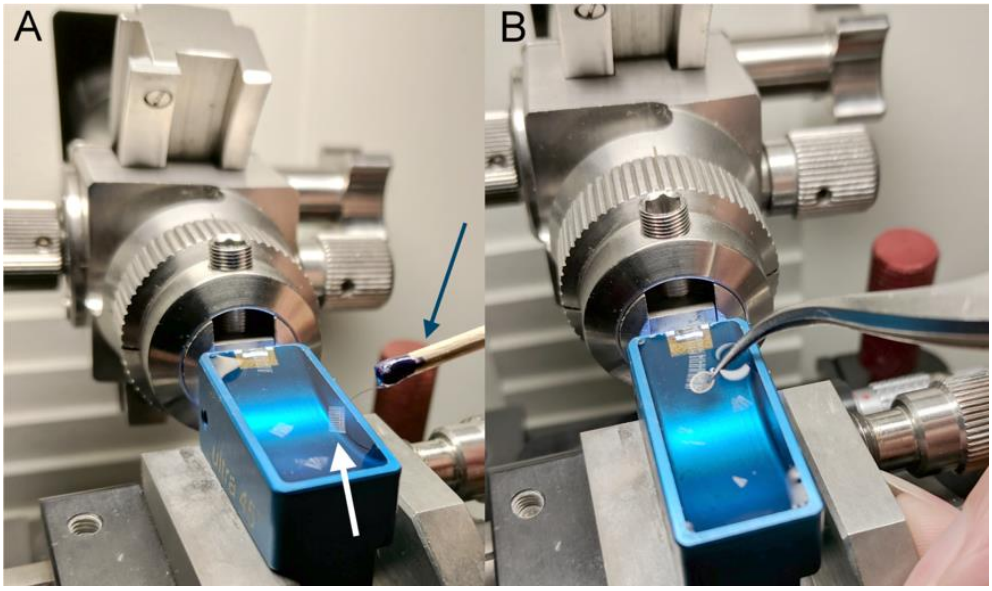

Figure 22: Manipulation of sections. *Sections (white arrow) being manipulated with an eyelash brush (blue arrow) (A). The sections are then collected onto a grid (B). Adopted from Hagler (2007).*

Before preparing the ultrathin sections, we surveyed semithin sections using a light microscope to determine whether the right area of the specimen is in a position for ultrathin sectioning. Once we were sure we are in the proper area of soldiers' heads where rostral gland could occur we send our samples for ultrathin sections preparation to a specialist at Charles University. Ultrathin sections were prepared on a Reichert-Jung Ultracut E ultramicrotome and stained using uranyl acetate and lead citrate. The

sections were placed on grid. Grid is a small (five millimetres in diameter) copper discs cast with a fine mesh. This mesh can vary a lot depending on the intended application but is usually about 15 squares per millimetre (400 squares per inch). Our grid was 300 Mesh slim bar copper made by SPI company.

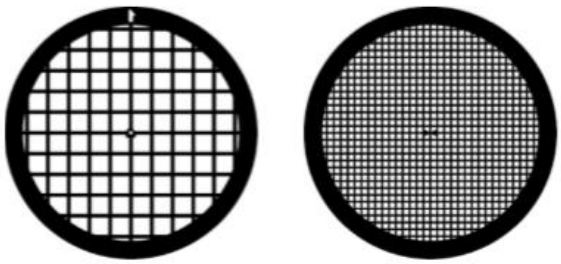

Figure 23: *Overhead view of a grid, showing the mesh. Adopted from Kubínek* et al. *(2011).*

4.1.3. Transmission electron microscopy (TEM)

Electron microscopy (EM) is a technique for obtaining high resolution images of biological or non-biological samples. EM is a versatile tool with a range of methodologies to characterize the microstructural features of a sample from 100pm to 100μm length scales. This technique is an important analytic tool in biology to investigate the detailed structure of tissues, cells, organelles and macromolecular complexes. The images from electron microscope are results from the use of electrons (which have very short wavelengths) as the source of illuminating radiation. EM images provide the key information on the structural basis of cell function, cell organelles and macromolecular complexes. There are two main types of electron microscopes – the transmission electron microscopy (TEM) and the scanning electron microscopy (SEM). The transmission electron microscope is used to observed thin samples (tissue sections, molecules, etc) through which electrons can pass generating a projection image. The TEM is analogous in many ways to the light microscope. TEM can be used to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture) (Wischnitzer, 2013). In our case we used transmission electron microscope to observe the rostral glands of *Verrucositermes* soldiers. We were looking for secretory and canal cells. Sections were examined and photographed using JEOL JEM-1011 electron microscope.

Glands measurements

All dimensions were measured using Nikon NIS Elements software. Pictures resulting from histology, semithin resin sections or TEM preparation were used for this purpose.

5 Results

The rostral gland in *Verrucositermes tuberosus* is located on head of soldiers. Its location is characterized by tubercles on its head, where series of canals open to the exterior. In following chapter I will list our TEM observation and measurements of secretory cells, canal cells, cell organelles and cuticle.

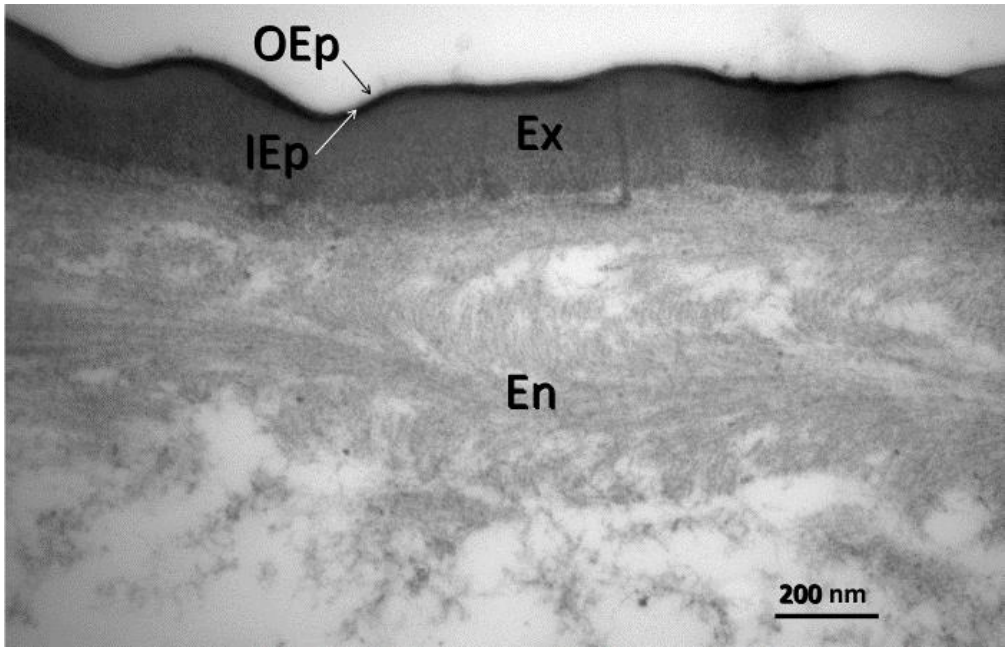

Figure 24: *Ultrastructure of non-sclerotised cuticle in soldiers of* Verrucositermes tuberosus. *Abbreviations: En – endocuticle, Ex – exocuticle, IEp – Inner epicuticle, OEp – Outer epicucticle,.*

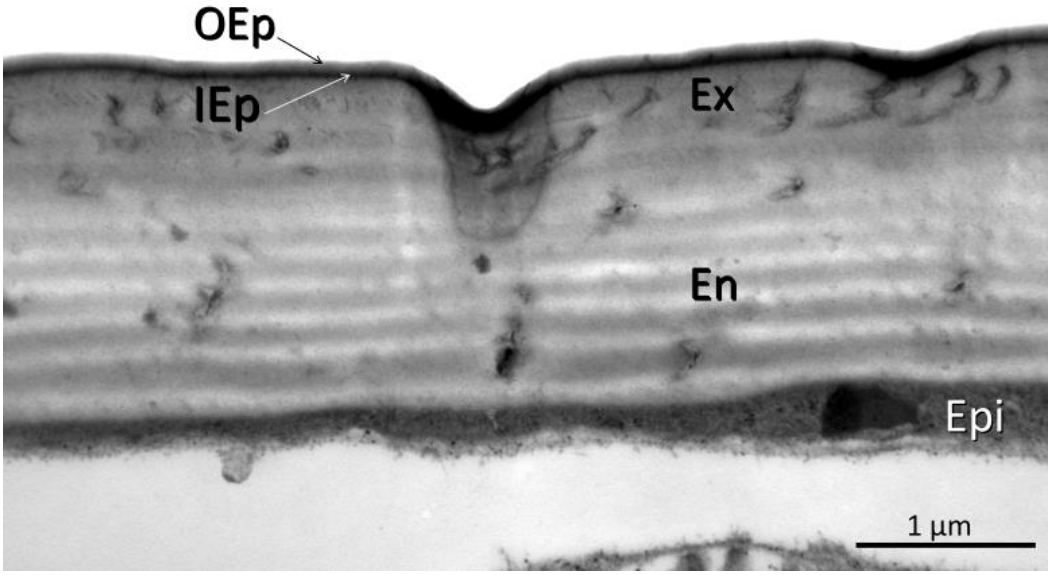

Figure 25: *Ultrastructure of sclerotised non - glandular cuticle and epidermal cell in soldiers of* Verrucositermes tuberosus. *Abbreviations: En – endocuticle, Epi-non modified epidermal cell, Ex – exocuticle, IOp – Inner epicuticle, OEp – Outer epicuticle.*

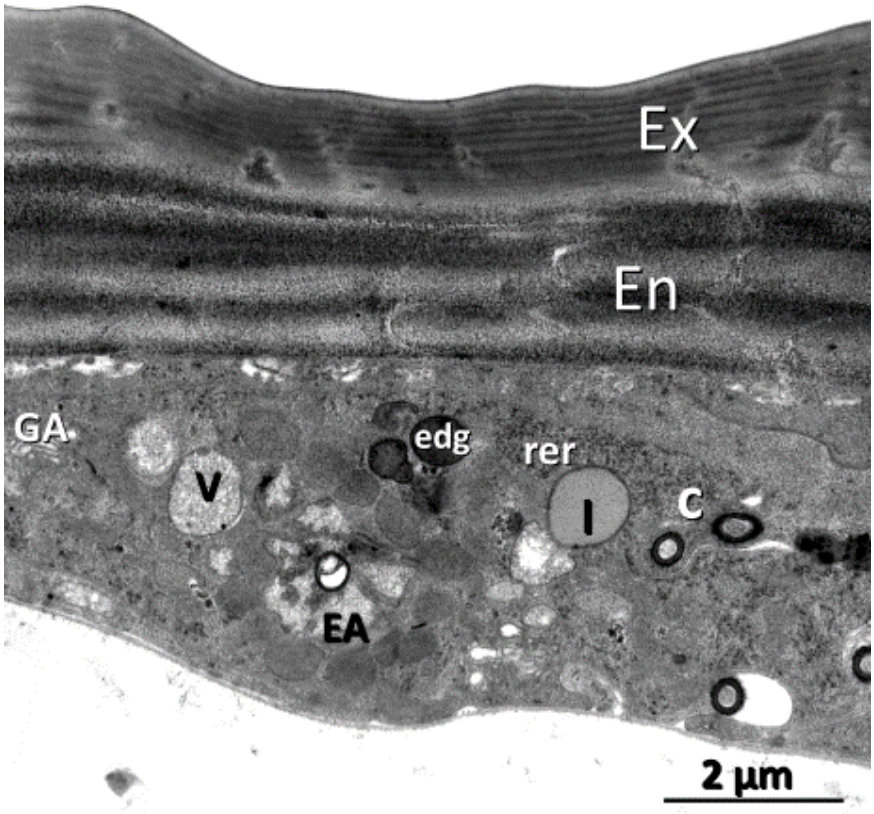

Figure 26: *Ultrastructure of secretory cells and cuticle in head of soldiers of* Verrucositermes tuberosus. *Abbreviations: c – canals, EA – end apparatus, edg – electron dense granule, En - endocuticle, Ex – exocuticle, GA – golgi apparatus, l – lipid-like droplet, rer – rough endoplasmatic reticulum, v – vesicle.*

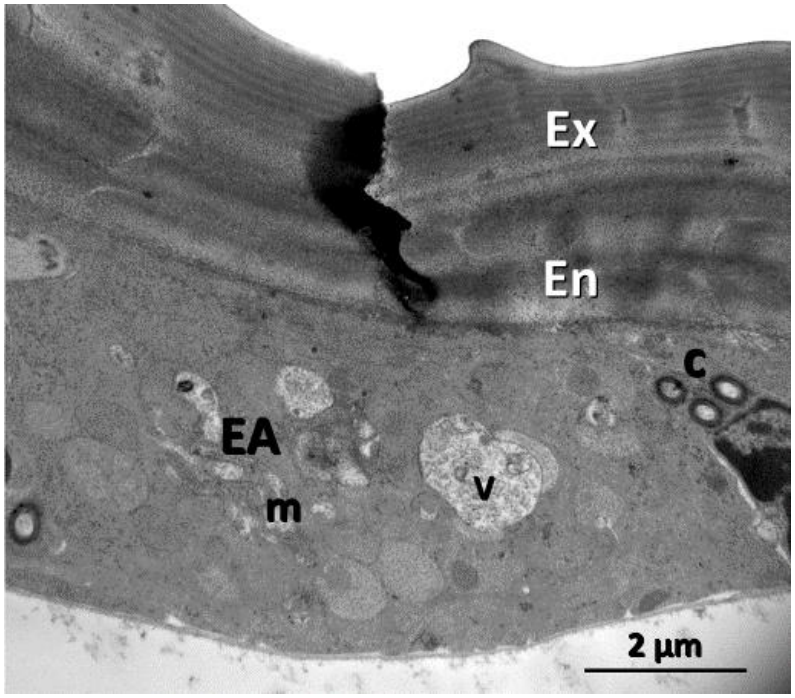

Figure 27: *Ultrastructure of secretory cells and cuticle in head of soldiers of* Verrucositermes tuberosus. *Abbreviations: c – canals, EA – end apparatus, En – endocuticle, Ex – exocuticle, m – microvilli, v – vesicle.*

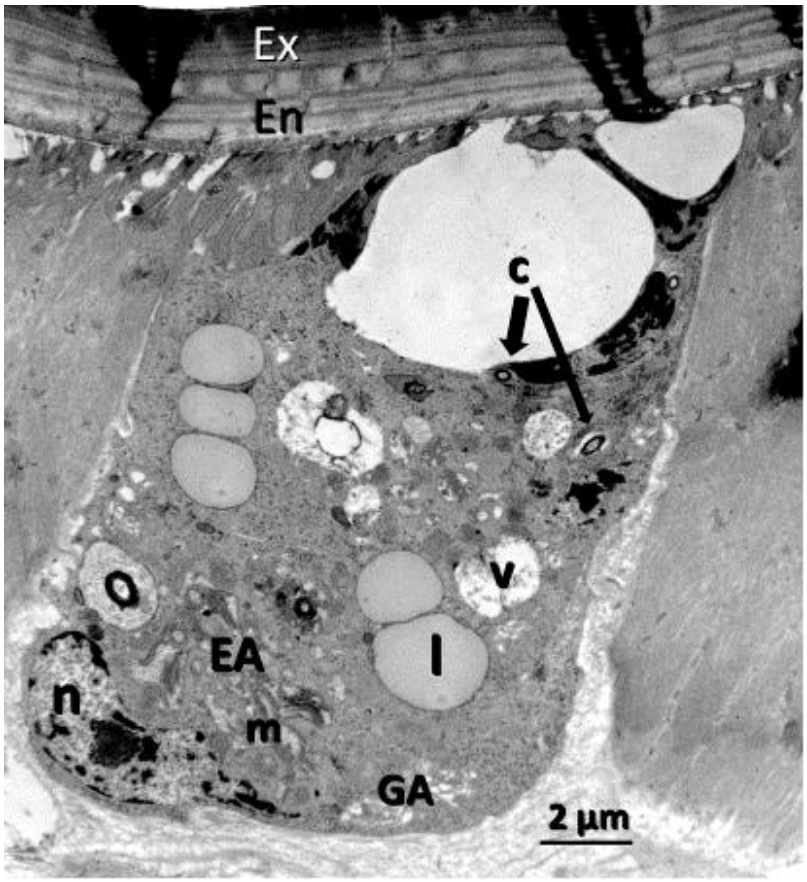

Figure 28: *Ultrastructure of secretory cells in head of soldiers of* Verrucositermes tuberosus. *Abbreviations: c – canals, GA – Golgi apparatus, En – endocuticle, Ex – exocuticle, l – lipid-like droplet, m – microvilli, n – nucleus, v – vesicle.*

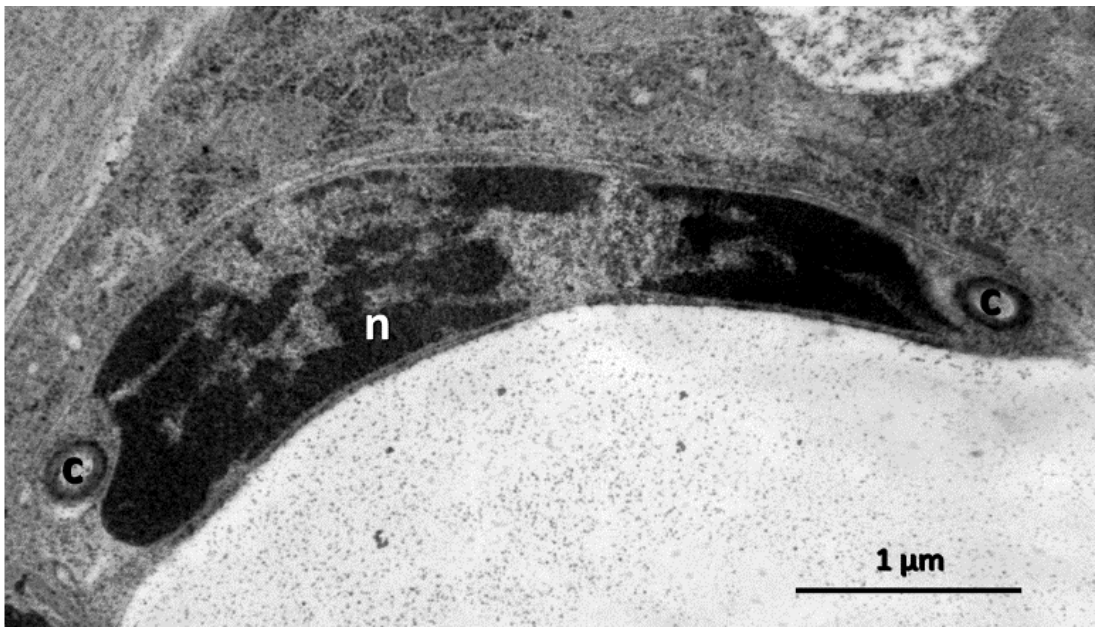

Figure 29: *Ultrastructure of canal cell in head of soldiers of* Verrucositermes tuberosus. *Abbreviations: c – canals, n – nucleus.*

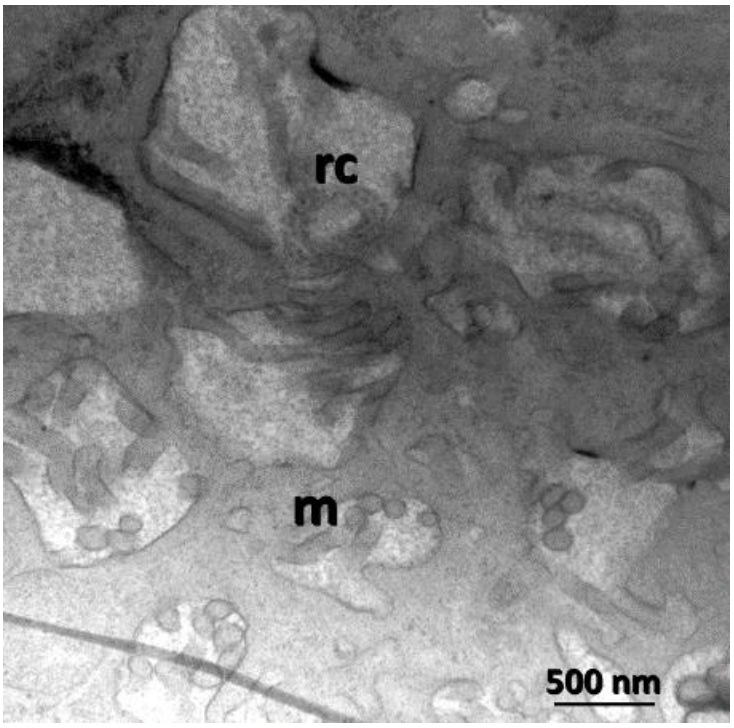

Figure 30: *Ultrastructure of end apparatus in secretory cell in head of soldiers of* Verrucositermes tuberosus. *Abbreviations: m – microvilli, rc – receiving canal.*

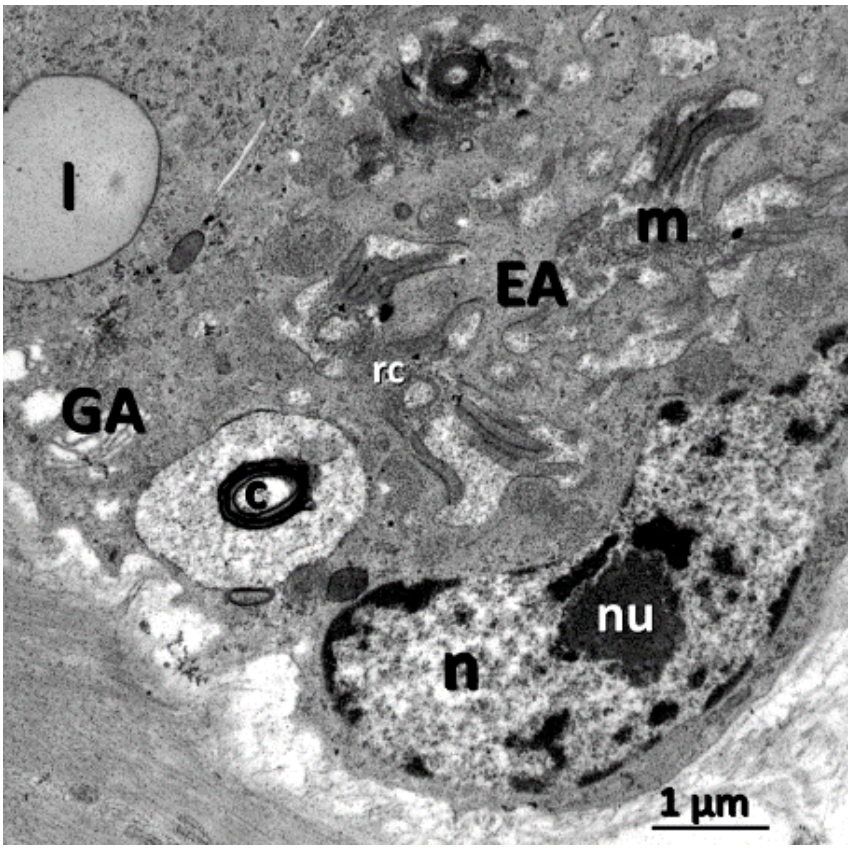

Figure 31: *Ultrastructure of secretory cell in head of soldiers of* Verrucositermes tuberosus. *Abbreviations: c – canal, GA – golgi apparatus, l – lipid like droplet, m – microvilli, n – nucleus, nu – nucleolus, rc – receiving canal.*

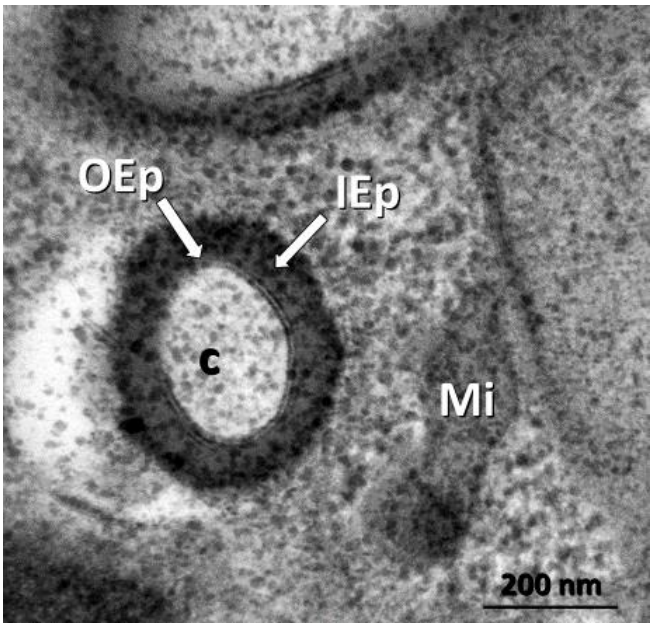

Figure 32: *Ultrastructure of canal in head of soldiers of* Verrucositermes tuberosus. *Abbreviations: c – canal, IEp – inner epicuticle, OEp – outer epicuticle.*

Measured data:

Table 1: Comparison of sizes of different modification of cuticle present in *Verrucositermes tuberosus.*

	Cuticle			
	Outer epic.	Inner epic.	Exocuticle	Endocuticle
Non-modified	≐ 8,5 nm	≐ 33 nm	≐ 195 nm	≐ 656 nm
Sclerotized non-glandular	≐ 15 nm	≐ 110 nm	≐ 583 nm	≐ 1,6 µm
Sclerotized glandular	Non-visible	≐ 45 nm	≐ 1,2 µm	≐ 1,8 µm

Table 2: Summary of measurements performed on secretory cells, canal cells, cell organelles and cuticle in *Verrucositermes tuberosus.*

	Width	Length
Microvilli	≐ 0,1 µm	≐ 0,7 µm
Canal	≐ 170 - 280 nm	n.a.
Lipid droplet	≐ 0,5 - 1,8 µm	n.a.
Glycogen	≐ 94 nm	n.a.
Golgi apparatus	≐ 0,6 - 2,3 µm	n.a.
End apparatus	≐ 3 µm	n.a.
Nucleus	≐ 6 µm	n.a.
Nucleolus	≐ 1 µm	n.a.
Canal cuticle	≐ 50 nm	n.a.
Secretory cell	≐ 7 µm	≐ 12 µm
Mitochondria	≐ 0,5 – 1,2 µm	n.a.
Vesicle	≐ 1,2 – 1,9 µm	n.a.
Electron dense granule	≐ 1 µm	n.a.

Soldiers of *Verrucositermes tuberosus* are equipped with numerous tubercules (cuticle outgrowths) on dorsal side of head and towards the nasus, and these correspond to outlets of class III secretory cells. The rostral gland has been observed after cross-sectioning this area. It is covered with sclerotized glandular cuticle and located beneath the non-modified epidermal cells. The comparison of non-modified epidermis and class III cells of rostral gland is on figures 25 and 26. Non-modified epidermis cells were small and inactive. Figures 24, 25, 26 and 27 show the comparison between non-sclerotised cuticle, sclerotized non-glandular cuticle and sclerotized glandular cuticle. All cuticles have the same morphology, they however differ in thickness and development of individual layers. Termite cuticle is formed by four different layers, from the top down namely (*i*) outer epicuticle, (*ii*) inner epicuticle, (*iii*) exocuticle and (*iv*) endocuticle. As you can see from table 1 the exocuticle and endocuticle were the thickest in the sclerotized glandular modification. We can observe a trend of thickening of individual layers towards the gland. The cuticle also forms the canals, which are made only of the epicuticular layers (Fig. 32). Average thickness of the canal cuticle was around 50 nm (table 2).

Rostral gland was formed by typical class III secretory cells (*sensu* Noirot & Quennedey, 1974), where a single secretory cell and corresponds to a single canal cell. The secretory cells had slightly columnar shape with average size 7 x 12 μm (table 2). They were significantly enlarged compared to non-modified epidermis cells. The enlargement relates to their higher activity. The nuclei were located in the cell base. Nuclear chromatin was loose due to ongoing transcription and with visible nucleolus. All secretory cells were rich in cell organelles connected to high secretory activity. Mitochondria, which are semi-autonomous double-membrane-bound organelle, ranged in size usually between 0,5 and 1 μm (Fig. 32, table 2). Rough endoplasmic reticulum is another common secretory organelles observed in every rostral gland secretory cell (Fig. 26). Golgi apparatus, which is part of the endomembrane system in the cytoplasm, was fairly common in all secretory cells. The size of Golgi apparatus in the secretory cells was from 0,6 to more than 2 μm (Fig. 26, 28, 31, table 2).

We have additionally observed secretory vesicles (Fig. 27, 28) and electron-dense granules (Fig. 26). Vesicles were little on average around 1,5 μm in diameter large, while electron-dense granules on average around 1 μm in diameter (table 2). The cytoplasm contained also free lipid-like droplets (Fig. 28, 31) with diameter between 0,5 and 1,8 μm and low amounts of smooth endoplasmic reticulum. The secretory

products in vesicles accumulated around the end apparatus, and rarely fuse with the apical plasma membrane. Average size of end apparatus in diameter was 3 μm (table 2). End apparatus was lined with microvilli, and a porous receiving canal is always inserted into it (Figs 26, 27, 28, 30 and 31). Average width of microvilli was 0,1 μm and length around 0,7 μm (table 2). Apart from the general structure described above the rostral gland cells contained many glycogen granules of average size around 94 nm in diameter.

The canal cells support the canals transporting the secretion to the exterior. Each canal consists of two parts, the receiving and the conducting canal. The conducting canal is solid whilst the receiving canal is porous. The receiving canal starts in the end apparatus, where through its pores scoops up the secretion. The conducting canal is solid because it provides a transport of secretion to its final destination (Noirot & Quennedy, 1991). The conducting canal can be observed on figures 26, 27, 28, 29, 30, 31 and 32. The pores of receiving canal can be observed on figures 30 and 31. Canal cell is largely inactive – it doesn't produce any own secretion, as shown at figure 29, where we can see condensed nucleus and no active organelles in cytoplasm. The average width of canals was between 170 – 280 nm (table 2).

5 Discussion

We confirmed the presence and described the ultrastructure of the rostral gland in soldiers of *Verrucositermes tuberosus* (Termitidae: Nasutitermitinae). The structure was before observed only in *Verrucositermes hirtus* using scanning electron microscope what disallowed any judgement on the functional significance of the tubercules, we conducted a detailed TEM study confirming the glandular nature of the tubercule-related tissue. The gland is composed of class III secretory cells accompanied by their respective canal cells. Those cells form together a bicellular glandular unit able to produce certain secretion and transport it to the exterior – on the surface of cephalic capsule. The function of the gland remains unknown, we can however speculate about its defensive function due to its position. It has been suggested that such glands occurring on the head of Nasutitermitinae (Termitidae) play an important role in preventing soldiers from getting stuck in their own sticky secretion originated from the frontal gland (Deligne *et al*., 1981).

Functional significance of the observed ultrastructure. Mitochondria are powerhouses of the cell. They generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. Golgi apparatus is an organelle used for packaging proteins into membrane-bound vesicles inside the cell before the vesicles are sent to their destination. It is of high importance in processing proteins for secretion. Rough endoplasmic reticulum is connected to the synthesis and export of proteins. The surface of the rough endoplasmic reticulum is studded with protein-manufacturing ribosomes giving it a "rough" appearance (Pollar *et al.*, 2017). End apparatus is a structure in cytoplasm. It is formed by reservoir with membrane formed by microvilli and porous receiving canal (Noitrot & Quennedy, 1991). Microvilli are microscopic cellular membrane protrusions that increase the surface area for diffusion and minimize any increase in volume. Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in animal (Pollar *et al.*, 2017).

General classification of termites glands is based on their ultrastructural features: (*i*) glands made of class I cells such as frontal, labral, and sternal glands in *Mastotermes* (Mastotermitidae) (Ampion and Quennedey, 1981; Quennedey, 1984; Quennedey et al., 2008; Šobotník et al., 2004, 2010b; Costa- Leonardo and Haifig, 2014), (*ii*) glands made of class III cells such as mandibular base glands in

Machadotermes (Termitidae: Apicotermitinae) and mandibular, lateral thoracic, tarsal, tergal, and pleural glands (Lambinet, 1959; Ampion, 1980; Ampion and Quennedey, 1981; Quennedey, 1984; Costa-Leonardo and Haifig, 2010, Gonçalves *et al*., 2010), (*iii*) glands made of class I and II cells such as sternal and posterior sternal glands (Ampion and Quennedey, 1981; Quennedey *et al*., 2008), (*iv*) glands made of class I and III cells such as frontal gland in *Coptotermes* and *Heterotermes* (both Rhinotermitidae), labral gland in *Glossotermes* (Serritermitidae), and clypeal, tergal, and tegumental glands (Ampion and Quennedey, 1981; Quennedey, 1984; Šobotník *et al.*, 2003, 2010b; Křížková *et al.*, 2014), (*v*) glands made of cells I, II and III class such as sternal, posterior sternal, and tergal glands (Ampion and Quennedey, 1981; Quennedey *et al.*, 2008; Šobotník *et al.*, 2005). In a few rare cases, however, this classification is impossible because the secretory cell type is unclear. The rostral gland is formed by class III cells accompanied with canal cells. This ultrastructure puts rostral gland in the second group along mandibular base glands in *Machadotermes* (Termitidae: Apicotermitinae) and mandibular, lateral thoracic, tarsal, tergal, and pleural glands.

The fixation protocol has proved to be suitable for the purpose. Although we had some troubles with sectioning the samples, we could unequivocally show the glandular nature of the tissue connected to the cephalic tubercules. This work is amongst the first steps we did on this way, and we will do our best to publish the results in a specialised structural journal, such as *Arthropod Structure & Development*, in the future.

6 References

Abe, T. (1987). Evolution of life-types in termites. In Kawano, S., Conell, J.H. & Hidaka, T. (Eds.). *Evolution and coadaptation in biotic communities* (pp. 125-148). University of Tokyo Press, Tokyo, Japan.

Ampion, M. (1980). *Les glandes tergales des imagos de termites: étude comparative et signification évolutive*. Phd Thesis. University of Dijon, France, 107 pp.

Ampion, M. & Quennedey, A. (1981). The abdominal epidermal glands of termites and their phylogenetic significance. *Systematics Association,19*, 249-261.

Austin, J.W., Szalanski, A.L., & Cabrera, B.J. (2004). Phylogenetic analysis of the subterranean termite family Rhinotermitidae (Isoptera) by using the mitochondrial cytochrome oxidase II gene. *Annals of the Entomological Society of America*, *97*(3), 548-555.

Bacchus, S. (1979). New exocrine gland on the legs of some Rhinotermitidae (Isoptera). *International Journal of Insect Morphology and Embryology*, *8*(2), 135-142.

Bacetti, B., & Dallai, R. (1978). The first multi-flagellate spermatozoa in the animal kingdom, discovered in *Mastotermes darwiniensis*. *Comptes rendus hebdomadaires des seances de l'Academie des sciences. Serie D: Sciences naturelles*, *285*(7), 785-788.

Barbosa, J.R.C., & Constantino, R. (2017). Polymorphism in the neotropical termite *Serritermes serrifer*. *Entomologia Experimentalis et Applicata*, *163*(1), 43-50.

Bignell, D. E., Eggleton, P., Nunes, L., & Thomas, K. L. (1997). Termites as mediators of carbon fluxes in tropical forest: Budgets for carbon dioxide and methane emissions. In A. D. Watt, N. E. Stork, & M. D. Hunter (Eds.), *Forests and insects* (pp. 109–134). London: Chapman & Hall.

Bignell, D. E., & Eggleton, P. (2000). Termites in ecosystems. In T. Abe, D. E. Bignell, & M. Higashi (Eds.), *Termites: Evolution, sociality, symbiosis, ecology* (pp. 363–387). Dordrecht: Kluwer Academic Publisher.

Bignell D.E. (2006) Termites as Soil Engineers and Soil Processors. In: König H., Varma A. (eds) *Intestinal Microorganisms of Termites and Other Invertebrates. Soil Biology*, vol 6. Springer, Berlin, Heidelberg

Bignell D.E., Roisin Y. & Lo N. (2011): *Biology of Termites: A Modern Synthesis*. New York: Springer, 576 pp.

Bignell, D.E. (2016). The role of symbionts in the evolution of termites and their rise to ecological dominance in the tropics. In Hurst, C.J (Ed.).*The Mechanistic Benefits of Microbial Symbionts* (pp. 121-172). Springer, Cham, Germany.

Billen, J., Joye, L. & Leuthold, R.H. (1989). Fine structure of the labial gland in *Macrotermes bellicosus* (Isoptera, Termitidae). *Acta zoologica*, *70*(1), 37-45.

Billen, J. & Šobotník, J. (2015). Insect exocrine glands. *Arthropod structure & development*, *44*(5), 399-400.

Bourguignon, T., Šobotník, J., Hanus, R. & Roisin, Y. (2009). Developmental pathways of *Glossotermes oculatus* (Isoptera, Serritermitidae): at the cross-roads of worker caste evolution in termites. *Evolution & development*, *11*(6), 659-668.

Bourguignon, T., Lo, N., Cameron, S.L., Šobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T. & Evans, T. A. (2015). The evolutionary history of termites as inferred from 66 mitochondrial genomes. *Molecular biology and evolution, 32*(2), 406-421.

Bourguignon, T., Lo, N., Šobotník, J., et al. (2017). Mitochondrial phylogenomics resolves the global spread of higher termites, ecosystem engineers of the tropics. *Molecular Biology and Evolution, 34*(3), 589-597.

Brossut, R. (1973). Evolution du système glandulaire exocrine céphalique des Blattaria et des Isoptera. *International Journal of Insect Morphology and Embryology, 2*(1), 35-54.

Cancello, E.M., Silva, R. R., Vasconcellos, A., Reis, Y.T., & Oliveira, L.M. (2014).Latitudinal variation in termite species richness and abundance along the Brazilian Atlantic Forest hotspot. *Biotropica, 46*, 441-450.

Cassier, P., Fain-Maurel, M.A. & Lebrun, D. (1977). Electron microscopic study of the mandibular glands of *Kalotermes flavicollis* Fabr. (Isoptera; Calotermitidae). *Cell and tissue research, 182*(3), 327-339.

Collins N.M., The utilization of nitrogen resources by termites (Isoptera). in: J.A. Lee, S. McNeill, I.H. Rorison (Eds.). (1983), Nitrogen as an Ecological Factor. *Blackwell Scientific Publications*, Oxford, pp. 381e412.

Constantino, R. & Costa-Leonardo, A.M. (1997). A new species of *Constrictotermes* from Central Brazil with notes on the mandibular glands of workers (Isoptera: Termitidae: Nasutitermitinae). *Sociobiology*, 213-223.

Costa-Leonardo, A.M., & Barsotti, R.C. (1996). Soldier head morphology of the Neotropical termites: *Embiratermes festivellus* Silvestri and *Spinitermes brevicornutus* (Desneux)(Isoptera, Termitidae). *Revista Brasileira de Zoologia, 13*(2), 321-330.

Costa-Leonardo, A.M., & Haifig, I. (2010). MRT letter: A novel tegumental gland in female imagoes of the neotropical termite *Cornitermes cumulans* (Isoptera, termitidae, syntermitinae). *Microscopy research and technique, 73*(11), 1005-1008.

Costa-Leonardo, A.M., & Haifig, I. (2014). Labral gland in soldiers of the neotropical termite *Cornitermes cumulans* (Isoptera: Termitidae: Syntermitinae). *Micron, 64*, 39-44.

Delattre, O., Sillam-Dussès, D., Jandák, V., Brothánek, M., Rücker, K., Bourguignon, T., Vytisková, B., Cvačka, J., Jiříček, O. & Šobotník, J. (2015). Complex alarm strategy in the most basal termite species. *Behavioral ecology and sociobiology, 69*(12), 1945-1955.

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

Deligne, J. (1983). Description, développement et affinités de *Verrucositermes hirtus* sp. n. Fonction glandulaire des tubercules du soldat (Isoptères, Nasutitermitinae). *Revue de zoologie africaine, 97*(3), 533-548.

Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J. (1994). Carbon Pools and flux of global forest ecosystems. *Science 263*:185–190.

Donovan SE, Eggleton P, Bignell DE (2001) Gut content analysis and a new feeding group classification of termites. *Ecol Entomol* 26:356–366

Eggleton P, Bignell DE, Sands WA, Mawdsley NA, Lawton JH, Wood TG, Bignell NC. (1996). The diversity, abundance and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Cameroon. *Philos Trans R Soc Lond B* 351:51–68.

Eggleton, P. (2000). Global patterns of termite biodiversity. In Abe, T., Bignell, D.E., Higashi, M. (Eds.). *Termites: evolution, sociality, symbioses, ecology* (pp. 25-52). Springer, Dordrecht, Netherlands.

Eggleton, P., Beccaloni G. and Inward D. (2007). Response to Lo et al. *Biology letters*. 3(5), 564-565.

Eggleton P. (2010) An Introduction to Termites: Biology, Taxonomy and Functional Morphology. In: Bignell D., Roisin Y., Lo N. (eds) *Biology of Termites: a Modern Synthesis*. Springer, Dordrecht

Eggleton, P. (2011): An Introduction to Termites: Biology, Taxonomy and Functional Morphology. In Bignell D.E., Roisin Y. & Lo N. (Eds): *Biology of Termites: A Modern Synthesis* (pp. 1-26). Springer, Dordrecht, Netherlands.

Engel MS, Grimaldi DA, Krishna K. (2007). Primitive termites from the early cretaceous. *Stuttgarter Beitrage Zur Naturkd 371*:1–32.

Engel MS, Grimaldi DA, Krishna K. (2009). Termites (Isoptera): their phylogeny, classification, and rise to ecological dominance. *Am Mus Novit. 3650*:1–27.

Emerson, A. E. (1936). Distribution of termites. *Science, 83*: (2157), 410.

French, J. R. J. (1988). A case for ecosystem-level experimentation in termite research. *SocioEconomic Planning Sciences, 14,* 269–280.

Gay, F.J., & Calaby, J.H. (1970). Termites of the Australian Region. InKrishna, K. & Weesner, F.M. (Eds.). *Biology of Termites, vol. II* (pp. 393-448). Academic Press, New York, USA.

Gonçalves, T.T., DeSouza, O. & Billen, J. (2010). A novel exocrine structure of the bicellular unit type in the thorax of termites. *Acta Zoologica, 91*(2), 193-198.

Grassé, P.P. (1982). *Termitologia. Volume 1: Anatomy, physiology and reproduction of termites* (pp. 123). Masson, Paris, France.

Grassé, P.P. (1984). *Termitologia. Tome II. Fondation des societes, construction*. Masson, Paris.

Grassé, P.P. and Noirot, C. (1951). La sociotomie: migration et fragmentation de la termitiere chez les Anoplotermes et les Trinervitermes. *Behaviour 3*, 146- 166

Greenberg, S.L., & Plavcan, K.A. (1986). Morphology and chemistry of the mandibular gland complex in the primitive termite, *Zootermopsis angusticollis* (Hagen)(Isoptera: Hodotermitidae). *International Journal of Insect Morphology and Embryology*, *15*(4), 283-292.

Grimaldi, D.A., & Engel, M.S. (2008). A termite bug in Early Miocene amber of the Dominican Republic (Hemiptera: Termitaphididae). *American Museum Novitates*, *2008*(3619), 1-10.

Grube, S. & Rudolph, D. (1999a). The labial gland reservoirs (water sacs) in *Reticulitermes santonensis* (Isoptera: Rhinotermitidae): studies of the functional aspects during microclimatic moisture regulation and individual water balance. *Sociobiology, 33, 307–323*.

Holmgren, N. (1909). Termitenstudien. I. Anatomische Untersuchungen. IX. Die Ernährungsorgane. *Kungliga Svenska Vetenskapsakademiens Handlingar, 44*.

Hagler, H.K. (2007). Ultramicrotomy for biological electron microscopy. *Methods Mol Biol*, 369:67-96.

Haverty MI, Howard RW (1981) Production of soldiers and maintenance of soldier proportions by laboratory experimental groups of Reticulitermes flavipes (Kollar) and Reticulitermes virginicus (Banks) (Isoptera: Rhinotermitidae). *Insectes Soc* 28:32–39

Hu J, Zhong JH, Guo MF. (2007). Alate dispersal distances of the blackwinged subterranean termite Odontotermes formosanus (Isoptera: Termitidae) in southern China. *Sociobiology* 50:1–8.

Inoue, T., Takematsu,Y., Yamada, A., Hongoh, Y., Johjima, T., Moriya, S., Sornnuwat, Y., Vongkaluang, C., Ohkuma, M., & Kudo, T. (2006). Diversity and abundance of termites along an altitudinal gradient in Khao Kitchagoot National Park, Thailand. *Journal of Tropical Ecology*, 22: 609-612.

Inward, D., Beccaloni, G., & Eggleton, P. (2007). Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biology letters*, *3*(3), 331-335.

Jones, D.T., & Eggleton, P. (2000). Sampling termite assemblages in tropical forests: Testing a rapid biodiversity assessment protocol. *Journal of Applied Ecology*, 37, 191–203.

Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P. & Lepage, M. (2006). Soil invertebrates as ecosystem engineers: intended and accidental effects on soil and feedback loops. *Applied soil ecology*, *32*(2), 153-164.

Jouquet, P., Traore, S., Choosai, C., Hartmann, C., & Bignell, D. (2011). Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *European Journal of Soil Biology*, 47, 215–222.

Kaib, M. & Ziesmann, J. (1992). The labial gland in the termiteS*chedorhinotermes lamanianus* (Isoptera: Rhinotermitidae): Morphology and function during communal food exploitation. *Insectes Sociaux*, *39*(4), 373-384.

Kambhampati S. & Eggleton P. (2010). Taxonomy and Phylogeny of Termites. Termites: Evolution, Sociality, Symbioses, Ecology. *Dordrecht: Springer Netherlands*, 1-23 pp.

Korb J, Linsenmair KE (2000) Ventilation of termite mounds: new results require a new model. *Behav Ecol 11*:486–494

Krishna K.. (1969). Biology of termites. S.l.: *Academic Press, New York, 597* pp.

Krishna K, Grimaldi DA. (2003). The first Cretaceous Rhinotermitidae (Isoptera): a new species, genus, and subfamily in Burmese amber. *Am Mus Novit. 3390*:1–10.

Krishna K, Grimaldi DA, Engel MS. (2013). Treatise on the Isoptera of the World: *Vol. 1. Bull Am Museum Nat Hist. 377*:1–196.

Křížková, B., Bourguignon, T., Vytisková, B. & Šobotník, J. (2014). The clypeal gland: a new exocrine gland in termite imagoes (Isoptera: Serritermitidae, Rhinotermitidae, Termitidae). *Arthropod structure & development*, *43*(6), 537-542.

Kubínek, R., Šafářová, K., Vůjtek, M. (2011). Elektronová mikroskopie. Univerzita Palackého v Olomouci, 1. edition, ISBN 978- 80-244-2739-3.

Kutalová, K., Bourguignon, T., Sillam-Dussès, D., Hanus, R., Roisin, Y. & Šobotník, J. (2013). Armed reproductives: evolution of the frontal gland in imagoes of Termitidae. *Arthropod structure & development*, *42*(4), 339-348.

Lambinet, F. (1959). La glande mandibulaire du termite â cou jaune (*Calotermes flavicollis*). Insectes sociaux, 6(2), 165-177.

Lepage, M., & Darlington, J.P.E.C., (2000). Population dynamics of termites. In: Termites: Evolution, Sociality, Symbioses, Ecology (Abe T., Bignell D.E. and Higashi M., Eds), *Kluwer Academic Publishers:* Dordrecht, The Netherlands. pp 333–361

Lo N, Tokuda G, Watanabe H, Rose H, Slaytor M, Maekawa K, Bandi C, Noda H. (2000). Evidence frommultiple gene sequences indicates that termites evolved from wood-feeding cockroaches. *Curr Biol. 10*:801–804.

Lo, N., T. Beninati, F. stone, et al. (2007). Cockroaches that lack Blattabacterium endosymbionts: the phylogenetically divergent genus Nocticola. *Biology letters. 3*(3), 327-330.

Moore, B.P. (1968). Studies on the chemical composition and function of the cephalic gland secretion in Australian termites. *Journal of Insect Physiology*, *14*(1), 33-39.

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

Noirot, C., Noirot-Timothee, C. and Han, S.H. (1986). Migration and nest building in *Cubitermes fungifaber. Insectes Sociaux 33*,361-374.

Noirot, C., & Pasteels, J.M. (1987). Ontogenetic development and evolution of the worker caste in termites. *Experientia*, *43*(8), 851-860.

Noirot, C., & Quennedey, A. (1991). Glands, gland cells, glandular units: some comments on terminology and classification. *Annales de la Société entomologique de France, 27*(2), 123-128.

Norkrans B. (1963). Degradation of cellulose. *Annu Rev Phytopathol*. 1:325–350.

Ohkuma M. & Brune A. (2011). Diversity, structure, and evolution of the termite gut microbial community. In: *Biology of termites: A modern synthesis*. Springer: 413-438 pp.

Pasteels J.M., (1968). Le système glandulaire tégumentaire des Aleocharinae (Coleoptera, Staphylinidae) et son évolution chez les espèces termitophiles du genre Termitella. — *Archs. Biol. (Liège),* 79: 381-469

Palin, O.F., Eggleton, P., Malhi, Y.C., Girardin, A.J., RozasDa´vila, A. & Parr, C.L. (2011). Termite diversity along an Amazon–Andes elevation gradient, Peru. *Biotropica*, 43: 100- 107.

Palma-Onetto, V., Hošková, K., Křížková, B., Krejčířová, R., Pflegerová, J., Bubeníčková, F., Plarre, R., Dahlsjö, C.A.L., Synek, J., Bourguigon, T., Sillam-Dussès, D. & Šobotník,

J. (2018). The labral gland in termite soldiers. *Biological Journal of the Linnean Society*, *123*(3), 535-544.

Palma-Onetto, V., Pflegerová, J., Plarre, R., Synek, J., Cvačka, J., Sillam-Dussès, D., & Šobotník, J. (2019). The labral gland in termites: evolution and function. *Biological Journal of the Linnean Society*, *126*(3), 587-597.

Percy J. (1979). Development and ultrastructureof sex-phenomone gland cells in females of the cabbage looper moth, Trichoplusia ni (Hübner) (Lepidoptera: Noctuidae). — *Canad. J. Zool.*, 57: 220-136.

Poiani, S.B., & 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.

Pollard, T. D., Earnshaw, W. C., Lippincott-Schwartz, J., & Johnson, G. T. (2017). *Cell biology*.

Prestwich, G.D. (1984). Defense mechanisms of termites. *Annual review of entomology*, *29*(1), 201-232.

Quennedey, A. (1984). Morphology and ultrastructure of termite defense glands. In Herman, H.R. (Ed.). *Defensive mechanisms in social insects* (pp. 151-200). Praeger, New York, USA.

Quennedey, A., Peppuy, A., Courrent, A., Robert, A., Everaerts, C. & Bordereau, C. (2004). Ultrastructure of posterior sternal glands of Macrotermes annandalei (Silvestri): 142

Quennedey, A., Sillam-Dussès, D., Robert, A. & Bordereau, C. (2008). The fine structural organization of sternal glands of pseudergates and workers in termites (Isoptera): a comparative survey. *Arthropod structure & development*, *37*(3), 168-185.

New members of the sexual glandular set found in termites (Insecta). *Journal of morphology*, *262*(3), 683-691.

Rahbek, C. (1995). The elevational gradient of species richness: a uniform pattern? *Ecography*, 18: 200-205.

Raina, A., Murphy, C., Florane, C., Williams, K., Park, Y.I. & Ingber, B. (2007). Structure of spermatheca, sperm dynamics, and associated bacteria in Formosan subterranean termite (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America*, *100*(3), 418-424.

Roisin, Y. (1990). Reversibility of regressive molts in the termite*Neotermes papua*. *Naturwissenschaften*, *77*(5), 246-247.

Roisin, Y. (2000). Diversity and evolution of caste patterns. In Abe, T., Bignell, D.E., Higashi, M. (Eds.). *Termites: evolution, sociality, symbioses, ecology*(pp. 95-120). Springer, Dordrecht, Netherlands.

Roisin, Y. & Korb, J. (2011). Social organisation and the status of workers in termites. In Bignell, D.E., Roisin, Y. & Lo, N. (Eds.). *Biology of termites: a modern synthesis* (pp. 133-164). Springer, Dordrecht, Netherlands.

Sbrenna, G., & Leis, M. (1983). Fine structure of the integumental glands of a termite soldier. *Tissue and Cell*, *15*(1), 107-119.

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

Šobotník, J. & Weyda, F. (2003). Ultrastructural ontogeny of the labial gland apparatus in termite *Prorhinotermes simplex* (Isoptera, Rhinotermitidae). *Arthropod structure & development*, 31(4), 255-270.

Šobotník, J., Weyda, F., Hanus, R., Kyjaková, P. & Doubský, J. (2004). Ultrastructure of the frontal gland in *Prorhinotermes simplex* (Isoptera: Rhinotermitidae) and quantity of the defensive substance. *European Journal of Entomology, 101*(1), 153-163.

Šobotník, J., Weyda, F. & Hanus, R. (2005). Ultrastructural study of tergal and posterior sternal glands in *Prorhinotermes simplex* (Isoptera: Rhinotermitidae). *European Journal of Entomology, 102*(1), 81-88.

Šobotník, J., Jirošová, A. & Hanus, R. (2010a). Chemical warfare in termites. *Journal of Insect Physiology, 56*(9), 1012-1021.

Šobotník, J., Sillam-Dussès, D., Weyda, F., Dejean, A., Roisin, Y., Hanus, R. & Bourguignon, T. (2010b). The frontal gland in workers of Neotropical soldierless termites. *Naturwissenschaften, 97*(5), 495-503.

Šobotník, J., Bourguignon, T., Hanus, R., Sillam-Dussès, D., Pflegerová, J., Weyda, F., Kutalová, K., Vytisková, B. & Roisin, Y. (2010c). Not onlysoldiers have weapons: evolution of the frontal gland in imagoes of the termite families Rhinotermitidae and Serritermitidae. *PLoS One, 5*(12), e15761.

Šobotník, J., Bourguignon, T., Hanus, R., Demianová, Z., Pytelková, J., Mareš, M., Foltynová, P., Preisler, J., Cvačka, J., Krasulová, J. & Roisin, Y. (2012). Explosive backpacks in old termite workers. *Science, 337*(6093), 436-436.

Šobotník, J., Kutalová, K., Vytisková, B., Roisin, Y. & Bourguignon, T. (2014). Age-dependent changes in ultrastructure of the defensive glands of *Neocapritermes taracua* workers (Isoptera, Termitidae). *Arthropod structure & development, 43*(20), 5e210.

Šobotník, J., Bourguignon, T., Carrijo, T.F., Bordereau, C., Robert, A., Křížková, B., Constantini, J.P. & Cancello, E.M. (2015). The nasus gland: A new gland in soldiers of *Angularitermes* (Termitidae, Nasutitermitinae). *Arthropod structure & development, 44*(5), 401-406.

Sreng, L. (1984). Morphology of the sternal and tergal glands producing the sexual pheromones and the aphrodisiacs among cockroaches of the subfamily Oxyhaloinae. *Journal of Morphology, 182,* 279–294.

Stuart, A., M. (1963). The structure and function of the sternal gland in Zootermopsis nevadensis (Isoptera). *Proceedings of the Zoological Society of London, 143, 43-52*.

Synek, J., Beránková, T., Stiblik, P., Pflegerová, J., Akama, P.D., Bourguignon, T., Silla-Dussès, D. & Šobotník, J. (2019). The oral gland, a new exocrine organ of termites. *Arthropod structure & development, 51*, 32-36.

Tůma, J., Fleiss, S., Eggleton, P., Frouz, J., Klimes, P., Lewis, O.T., Yusah, K.M., Fayle, T.M. (2019) Logging of rainforest and conversion to oil palm reduces bioturbator diversity but not levels of bioturbation. *Appl. Soil Ecol.*, 144, 123–133

Watson, J.A.L. & Gay, F.J. (1991). Isoptera (Termites). In Naumann, I.D., Came, P.B. (Eds.). *The Insect of Australia* (pp. 330-348). Melbourne University Press, Melbourne, Australia.

Weesner, F.M. (1969). External anatomy. In Krishna, K., Weesner, F.M. (Eds.).*Biology of termites, vol. I* (pp. 19-47). Academic press, New York, USA.

Willig, M.R., Kaufman, D.M., & Stevens, R.D. (2003). Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution and Systematics, 34*: 273-309.

Wilson E.O. (1971). *The Insect Societies*. Belknap Press, Cambridge, USA, 548 pp.

Wischnitzer, S. (2013). *Introduction to Electron Microscopy*, Elsevier Science, from https://books.google.cz/books?id=SZM4BQAAQBAJ.

Wu, L.W., Bourguignon, T., Šobotník, J., Wen, P., Liang, W.R. & Li, H.F. (2018). Phylogenetic position of the enigmatic termite family Stylotermitidae (Insecta: Blattodea). *Invertebrate systematics*, *32*(5), 1111-1117.

7 Supplementary material

Nomenclature of species and genera mentioned in the text of thesis. In alphabetical order, adopted from Krishna (2013)

Acanthotermes acanthothorax (Sjöstedt, 1898)
Angularitermes Emerson, 1925
Angularitermes nasutissimus (Emerson, 1925)
Aparatermes Fontes, 1987
Aparatermes cingulatus (Burmeister, 1839)
Apicotermes Holmgren, 1912
Archotermopsis wroughtoni (Desneux, 1904)
Armitermes grandidens (Emerson, 1925)
Coarctotermes suffuscus (Snyder, 1949)
Coptotermes Wasmann, 1896
Coptotermes formosanus (Shiraki, 1909)
Coptotermes sjostedti (Holmgren, 1911)
Cornitermes Wasmann, 1897
Cornitermes bequaerti (Emerson, 1952)
Cryptotermes havilandi (Sjostedt, 1900)
Cryptotermes verruculosus (Emerson, 1925)
Cubitermes Wasmann, 1906
Embiratermes Fontes, 1985
Embiratermes festivellus (Silvestri, 1901)
Glossotermes Emerson, 1950
Glossotermes oculatus (Emerson, 1950)
Heterotermes Froggatt, 1897
Jugositermes tuberculatus (Emerson, 1928)
Kalotermes Hagen, 1853
Kalotermes flavicollis (Fabricius, 1793)
Macrotermes Holmgren, 1910
Macrotermes carbonarius (Hagen, 1858)
Machadotermes Weidner, 1974
Mastotermes Froggatt, 1897
Mastotermes darwiniensis (Froggatt, 1897)
Microcerotermes Silvestri, 1901
Microcerotermes fuscotibialis (Sjostedt, 1896)
Nasutitermes Dudley, 1890
Nasutitermes octopilis (Banks, 1918)
Nasutitermes triodiae (Froggatt, 1898)
Neocapritermes Holmgren, 1912
Neocapritermes taracua (Krishna and Araujo, 1968)
Odontotermes Holmgren, 1912
Pericapritermes Silvestri, 1914
Pericapritermes urgens (Silvestri, 1914)
Porotermes Hagen, 1858
Procubitermes niapuensis (Emerson, 1928)
Promirotermes Silvestri, 1914
Promirotermes orthocopes (Emerson, 1928)
Prorhinotermes Silvestri, 1909

Prorhinotermes simplex (Hagen, 1858)
Psammotermes Desneux, 1902
Rhinotermes Hagen, 1858
Rhinotermes hispidus (Emerson, 1925)
Rugitermes bicolor (Emerson, 1925)
Ruptitermes Mathews, 1977
Serritermes Wasmann, 1897
Stolotermes Hagen, 1858
Tenuirostritermes Holmgren, 1912
Termes Linnaeus, 1758
Termitogeton Desneux, 1904
Verrucositermes Emerson, 1960
Verrucositermes tuberosus (Emerson, 1960)
Verrucositermes hirtus Deligne, 1983
Zootermopsis Emerson, 1933
Zootermopsis angusticollis (Hagen, 1858)