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RESEARCH

The Trail-Following Communication in *Stylotermes faveolus* and *S. halumicus* (Blattodea, Isoptera, Stylotermitidae)

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Abstract

Stylotermitidae appear peculiar among all termites, feeding in trunks of living trees in South Asia only. The difficulty to collect them limits the ability to study them, and they thus still belong to critically unknown groups in respect to their biology. We used a combination of microscopic observations, chemical analysis and behavioural tests, to determine the source and chemical nature of the trail-following pheromone of *Stylotermes faveolus* from India and *S. halumicus* from Taiwan. The sternal gland located at the 5th abdominal segment was the exclusive source of the trail-following pheromone in both *S. faveolus* and *S. halumicus*, and it is made up of class I, II and III secretory cells. Using gas chromatography coupled mass spectrometry, (*3Z*)-dodec-3-en-1-ol (DOE) was identified as the trail-following pheromone which elicits strong behavioural responses in workers at a threshold around 10^{-4} ng/cm and 0.1 ng/gland. Our results confirm the switch from complex aldehyde trail-following pheromones occurring in the basal groups to simpler linear alcohols in the ancestor of Kalotermitidae and Neoisoptera.

Keywords (*3Z*)-dodec-3-en-1-ol · sternal gland · Neoisoptera · Semiochemicals

Introduction

Termites are the most prominent decomposers at the global scale, with a total biomass comparable to ants, the other eusocial insect dominating the Earth (Bar-on et al. 2018; Tůma et al. 2020). The 3,000 known termite species belong to ten living families: Mastotermitidae, Hodotermopsidae, Archotermopsidae, Hodotermitidae, Stolotermitidae, Kalotermitidae, Stylotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae (Krishna et al. 2013; Wang et al. 2022), with Stylotermitidae being largely unknown in respect to their biology (Liang et al. 2017; Wu et al. 2018). A single extant genus in this family, *Stylotermes*, includes 45 described species living in Asia (India through Bangladesh, China, Taiwan to Malaysia; Krishna et al. 2013; Liang et al. 2017; Wu et al. 2018). *Stylotermes* is a sister group to all remaining Neoisoptera characterised by the presence of a frontal gland, and reveals unique combination of characters intermediate between basal and advanced taxa (Wu et al. 2018). *Stylotermes* is unique amongst all termites in many features, such as living inside and feeding on live woody tissues or having three tarsomeres (Roonwal and Chotani 1989; Wu et al. 2018). All other knowledge of its biology

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comprises a few anecdotal comments scattered through the specialised taxonomical literature (Krishna et al. 2013).

Pheromones are widely used in all social insects for the exchange of information and maintenance of social equilibrium in the colony, termites as well use a broad array of primer and releaser pheromones (Billen and Morgan 1998; Bordereau and Pasteels 2010; Mitaka and Akino 2021). Some of the primer pheromones inhibit soldier differentiation (Mitaka et al. 2017), development of secondary queens and also egg-marking (Matsuura et al. 2010; Yamamoto and Matsuura 2011), and the releaser pheromones, such as those mediating sex- attraction and mating (Bordereau et al. 2010; Sillam-Dussès et al. 2011), aggregation or nest-mate gathering (Mitaka et al. 2020), alarm to coordinate defensive activities (Sillam-Dussès et al. 2023), food-marking in workers (Reinhard et al. 2002), and trail-following pheromones (Sillam-Dussès et al. 2005, 2007, 2009a, 2011, 2020, 2021; Bordereau and Pasteels 2010; Wen et al. 2014).

The trail-following pheromones are keys for coordinating termite mass activities, especially recruiting and navigation on the foraging paths (Sillam-Dussès et al. 2005, 2007, 2009a, 2011, 2020, 2021; Bordereau and Pasteels 2010; Wen et al. 2014). The trail-following pheromones are produced by the sternal glands, located on the third, fourth and fifth abdominal sternites in *Mastotermes*, on the fourth sternite in Teletisoptera and on the fifth sternite in more advanced groups, like the Kalotermitidae and Neoisoptera (Quennedey et al. 2008). Trail-following pheromones represent the most widely studied pheromone category in termites (Mitaka and Akino 2021). Till date, only eight different compounds (aldehydes, alcohols, diterpenes, and ketones) have been identified as trail-following pheromones in more than 80 different species (Sillam-Dussès 2010, 2011; Sillam-Dussès et al. 2009a, 2020, 2021; Bordereau and Pasteels 2010; Wen et al. 2014). The pheromones in basal families consist of complex C14 or C18 skeletons, (*E*)-2,6,10-trimethyl-5,9-undecadien-1-ol (*Mastotermes*, *Porotermes*, *Stolotermes*; Sillam-Dussès et al. 2007), *syn*-4,6-dimethyldodecanal (*Zootermopsis*; Bordereau et al. 2010) and *syn*-4,6-dimethylundecan-1-ol (*Hodotermopsis*; Lacey et al. 2011). There is however an evolutionary switch to much simpler C12 alcohols in all more advanced groups (Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae), although other unrelated compounds may play the same role. (3*Z*)-Dodec-3-en-1-ol is the pheromone of two unrelated groups, Kalotermitidae (Sillam-Dussès et al. 2009a), and Termitidae: Macrotermitinae (Peppuy et al. 2001a, b; Robert et al. 2004; Wen et al. 2017). (3*Z*,6*Z*)-Dodeca-3,6-dien-1-ol is known only from Macrotermitinae (Peppuy et al. 2001a, b; Robert et al. 2004; Wen et al. 2017). (3*Z*,6*Z*,8*E*)-Dodeca-3,6,8-trien-1-ol (dodecatrienol) is the most common of all trail-following pheromones, known from all Rhinotermitidae and almost all Termitidae studied so far (Bordereau and Pasteels 2010; Sillam-Dussès et al. 2020, 2021). Surprisingly, diterpenic (C20) neocembrene is a minor or major component of trail-following pheromones, along with dodecatrienol in several unrelated groups, such as Rhinotermitidae: *Prorhinotermes* (Sillam-Dussès et al. 2005, 2009b), Termitidae: Termitinae: *Amitermes* (Kotoklo et al. 2010) or many Nasutitermitinae (Sillam-Dussès et al. 2010). Yet, another trail-following pheromone is C19 aldehyde, (10*Z*,13*Z*)-10,13-nonadecadien-2-one, known from Serritermitidae (Hanus et al. 2012; Sillam-Dussès et al. 2021).

While the trail-following pheromones have already been identified in all termite families except Hodotermitidae and Stylotermitidae, it is only the latter which remains considerably understudied, because of the paucity of materials and difficulty to study due to their nesting habits (within living tree trunks) (Krishna et al. 2013; Wu et al. 2018).

Even though this family remains understudied, it represents a crucial group to understand ecological shifts in the evolution of the advanced termites, and the nature of the trail-following pheromones is an important part of it. We have therefore sampled two *Stylotermes* species i.e., *S. faveolus* (Chatterjee and Thakur 1964) from India and *S. halumicus* Liang et al. (2017) from Taiwan, in order to determine the nature of the trail-following pheromone in this group.

Materials and Methods

Insects

Two colonies of *Stylotermes faveolus* (Chatterjee and Thakur 1964) (Blattodea: Isoptera: Stylotermitidae) were sampled at Hill Agricultural Research and Extension Centre, Bajaura, Kullu, Himachal Pradesh, India (N 31°50’11” E 77°10’23”, 1,050 m amsl) from *Populus deltoides* and *Alnus nitida* trees, respectively. Both groups contained about 500 hundred workers (probably pseudergates *sensu lato*; Roisin and Korb 2011) and tens of soldiers. *Stylotermes halumicus* Liang et al. (2017) was collected in a *Trema orientalis* (L.) tree located at Dakeshan Forest Recreation Area, Zhuolan Township, Miaoli County, Taiwan (N 24°19’45.5” E 120°53’45.6”, 650 m amsl) between September and November, 2022. The *S. halumicus* colony was found in a tree hole, which contained damp and mud-like substance. At least 50 individuals of the same *S. halumicus* colony were collected three times, and bioassays were conducted in three weeks.

Structure of the Sternal Gland

We used the well-established protocol described by Šobotník and Weyda (2003) and Šobotník et al. (2004). Semithin sagittal sections (0.5 µm) of worker abdomens were stained with 1% methylene blue and observed using optical compound microscope (Leica DMLB, Leica Microsystems, Germany) at All India Institute of Medical Sciences, New Delhi, India. The ultrathin sections (40–60 nm) were stained after Reynolds (1963), and observed using Thermo Scientific TECNAI G20 HR 200 kV Transmission electron microscope.

Pheromone Extraction

The sternal glands, located at the anterior medial part of the fifth abdominal sternite, were carefully dissected after cold anesthetized workers under Magnus MSZ-Bi (Olympus, India) stereomicroscope with microscissors and extracted with p.a. *n*-hexane for 12 h at 4 °C. Extracts were stored at − 20 °C until required for analysis. In total, glands of 669 *S. faveolus* workers were dissected for chemical analysis and bioassays.

Gas-Chromatography Coupled to Mass Spectrometry Analysis

Prior to instrumental analysis, sternal gland extracts intended for chemical analysis were evaporated to dryness under a gentle stream of argon. Afterwards, the residue was concentrated by adding 5 µl of *n*-hexane, containing 5 µg/ml of 1-bromododecane (as internal standard). One microliter was injected into the split/splitless injector in the splitless mode with the injection temperature at 275 °C; splitless period 120 s. For separation, detection and quantification purposes, a comprehensive two-dimensional gas chromatographic-mass spectrometric system Pegasus 4D (LECO, St. Joseph, MI, USA) employing Agilent 7890B was used. Separation was conducted on HP-5MS UI capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) coupled to VF-17MS (1.2 m, 0.1 mm i.d., 0.1 µm film thickness). Both columns were from Agilent (USA). The GC oven temperature was program as followed: 40 °C for 2 min; then ramped at a rate of 10 °C/min to 100 °C; then at 5 °C/min to 320 °C, and held at this temperature for 3 min. Second dimension oven and modulator had an offset of 5 and 15 °C respectively. Modulation period (consumable free modulator) was 5 s. The total GC run time was 55 min including 550 s solvent delay. Electron ionization (ionisation energy at 70 eV) mass spectra were collected in a mass range of 35–500 Da with a frequency of 100 Hz. (*Z*)-Dodec-3-en-1-ol (DOE) was identified in the extracts by comparing mass spectral and retention time information obtained from measurement of pheromone standard solutions. Quantification was performed using external calibration curve of DOE and internal standard.

Chemical Standards

The standard of (*Z*)-dodec-3-en-1-ol was synthesized by Martine Lettere (INRAe, Versailles, France) in 2008 (Sillam-Dussès et al. 2009a) and stored at -20 °C since then. Purity of the compound was 98– 99%. Standard of 1-bromododecane was obtained from Acros Organics (USA).

Trail-Following Bioassays

The work with *Stylotermes faveolus* was conducted at 25 °C and 60% RH at the Animal Plant Interactions Lab (Department of Zoology, Sri Venkateswara College, Delhi University, India). Similar studies for *S. halumicus* were conducted at the Department of Entomology, National Chung Hsing University, Taichung, Taiwan. The trail-following behaviour was tested using an "Y open field" bioassays on Whatman No. 1 filter paper. The filter paper was perforated to form a "Y" with holes spaced one cm apart. The stem of the "Y" was 3 cm long while the branches were 7 cm long and 120° angles separated them. On the Y stem (3 cm) and on one of the Y branches (7 cm), an artificial trail was drawn with a microliter syringe containing 1 µl of extract per 1 cm of trail. Another extract or hexane as a control was deposited in the same conditions on the stem and the other branch; left and right arms were changed randomly throughout the trials. The trails of extracts or hexane made this way evaporate a few seconds after being deposited, but the holes allow the trails to be seen. DOE solutions from 10^{-4} ng/cm to 1 ng/cm were used as stimuli, with hexane as a control, with 30 replications (for details see Sillam-Dussès et al. 2011 and Wen et al. 2014). One worker was deposited inside a waiting chamber made of a small plastic vial (55 mm in diameter), the narrow 2 mm wide opening being located at the Y stem. The distance travelled by the worker on the artificial trail (10 cm maximum) was the measure of trail-following activity elicited by the given solution. A new worker and a new filter paper were used each time to prevent any effects from trail reinforcement. The activity threshold was set to the minimum concentration that elicits termites to follow a mean distance of more than 3 cm (length of the Y stem, the maximum response being 10 cm). In the case of a choice test (DOE vs. gland extract), only the number of termites choosing any one of the trail or another was recorded, and the data were evaluated using a Monte Carlo test based on χ^2 test at the level of $p < 0.05$. Even though the termites could walk anywhere on the filter paper, they always followed one of the trails.

Results

Structure of the Sternal Glands

The sternal gland is located medially on the anterior part of the fifth abdominal sternite, overlaid with the previous one (Fig. 1). It is roughly 300 μm long and up to 60 μm thick (Fig. 2). The anterior part is dominated by class I secretory cells, among which a few secretory cells of classes II and III are squeezed in between. There is also a group of campaniform sensillae, which are attached to the cuticle in about one third of the gland length within the anterior portion. The posterior part is formed predominantly by class III secretory cells, but the part adjoining the cuticle is made of class I cells in substantially low volume. The cuticle overlying the gland, thick from 500 to 700 nm, is modified, facilitating the penetration of the secretion. The pore canals are enlarged and the epicuticle is pierced by numerous tiny pores.

Class I cells are columnar in the anterior part, while they are rather flat in the posterior part. The apical microvilli are 1.2 to 1.5 μm long and roughly 150 nm thick, and they are much denser in the anterior part compared to the posterior part. The nuclei (4 to 6 μm long) are ovoid, slightly irregular, filled with dispersed chromatin in class I cells, as well as in other two types of secretory cells. The cytoplasm contains copious amount of smooth endoplasmic reticulum, moderate amounts of secretory vesicles (about 0.5 μm in diameter) and large numbers of mitochondria. The junctions between neighbouring class I cells comprise apical zonula adherens followed by a long septate junction reaching to roughly a third or half of the secretory epithelium thickness; basal parts of the membranes are devoid of junctions. Class II secretory cells (up to 15 μm in the longest dimension) are embedded within the class I cells, and reach to the basement membrane only rarely. The cytoplasm contains predominantly rough endoplasmic reticulum, and rather rare Golgi apparatus releasing small electron-lucent vesicles. Class II cells also contain electron-dense granules of variable sizes (up to 5 μm) and sparce mitochondria. They do not reveal junctions with the neighbouring cells. Class III cells contain huge accumulations of electron-lucent vesicles, which pushed the cytoplasm into small islands amongst them. These islands contain only a few organelles, small mitochondria and rough endoplasmic reticulum located predominantly close to the nucleus. Small electron-dense granules were observed rarely. The end apparatus is formed by relatively few microvilli (800 nm long and 120 nm thick) surrounding a squeezed extracellular reservoir, into which the porous receiving canal (250–350 nm thick) is inserted. The canal cells are connected to the class III secretory cells by a short septate junction. They are always poor in cytoplasm and organelles, and contain flat nucleus made of condensed chromatin. The conducting canal is formed by epicuticle continuous with the body epicuticle, of which the outer epicuticle is about 15 nm thick and the inner epicuticle 30–35 nm thick. No free axons innervating the gland cells were observed.

Chemical Analyses

Chemical analyses of sternal gland extracts revealed a peak of DOE, slightly above limit of quantification, by comparing

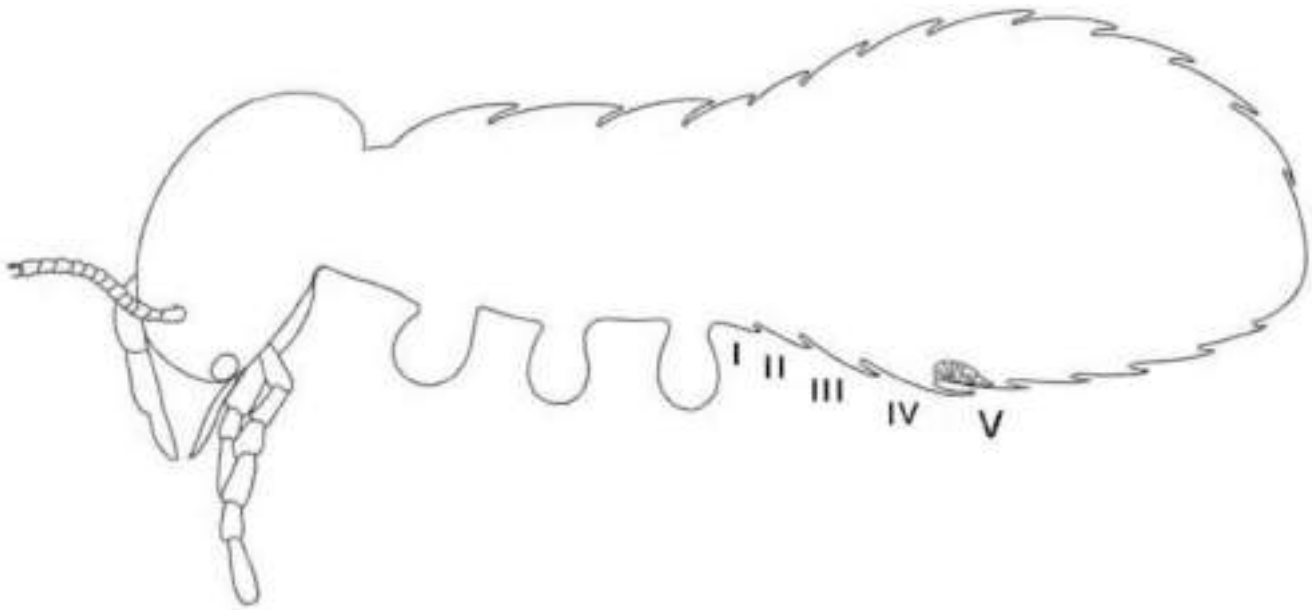

Fig. 1 Schematic drawing of a worker in *Stylotermes faveolus* or *S. halumicus* showing the position of the sternal gland. The number of the sternites are indicated in Roman numeral

the retention time and the characteristic masses of the standard. This compound was the only one of interest (Fig. 3). By measuring four pooled samples, we calculated the quantity of DOE per gland to be 0.1 ng (SD = 0.02 ng).

Trail-Following Bioassays

S. faveolus workers easily followed trails made of their own sternal gland extract as well as a broad range of DOE concentrations from 10^{-3} to 1 ng/cm. The activity threshold was at 10^{-3} ng/cm. At the highest concentration, workers could not follow trails. The control trails never elicited any trail-following behaviour (Table 1). Similar results were obtained with *S. halumicus*, but the sensitivity threshold was 10 times lower (Table 1). The comparison of sternal gland extracts and DOE at different concentrations in two-choice Y-open field trail-following bioassays suggest that the pheromone content ranges between 10^{-1} ng and 1 ng per one sternal gland in *S. faveolus* (Table 2). This corroborates the estimation made by chemical analysis.

Discussion

Termites are eusocial insects capable of mass activities such as foraging for food or colony defence, and these processes are regulated by the chemical communication among the colony-mates. One of the most important channels, the trail-following communication, is controlled by the sternal gland secretion (Bordereau and Pasteels 2010). Our study showed that *S. faveolus* (and likely *S. halumicus* as well) has a single sternal gland located on the fifth abdominal sternite, similarly to Kalotermitidae, Serritermitidae, Rhinotermitidae, and Termitidae (Noirot 1995; Quennedey et al. 2008). The gland is made of class I, II and III secretory cells occurring at comparable abundances. While the three classes are common in the sternal gland of Serritermitidae and Rhinotermitidae, only the classes I and II secretory cells have been observed in the sternal gland of Kalotermitidae and Termitidae (Quennedey et al. 2008). We may thus say that the presence of class III cells in the sternal gland is an apomorphy of Neoisoptera, which was secondarily lost in Termitidae. Our study demonstrated that the sternal gland of both *Stylotermes* species contains (*Z*)-dodec-3-en-1-ol (DOE) in quantity around 10^{-1} ng in *S. faveolus*. DOE elicits trail-following activity, and is therefore the trail-following pheromone of both *Stylotermes* species. The same compound is the trail-following pheromone also in Kalotermitidae (Sillam-Dussès et al. 2009a), what could be explained by a single evolutionary event in the common ancestor of Neoisoptera and Kalotermitidae, living around 130–140 mya (Buček et al. 2019). The more advanced groups started then using a structurally similar compound, (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol, but DOE was secondarily acquired in some more advanced species of the Termitidae family (Peppuy et al. 2001a; Wen et al. 2014). The quantity of DOE in *Stylotermes* is similar to that of Kalotermitidae (Sillam-Dussès et al. 2009a), and other Macrotermitinae, even though sometimes in association with another compound (Peppuy et al. 2001b; Wen et al. 2014, 2017). Interestingly, DOE is also known as female sex-pairing pheromone in *Cornitermes snyderi* (Termitidae: Syntermitinae; Bordereau et al. 2011). This shows a high degree of conservation during the evolution of termite pheromones, across species belonging to different families (Bordereau and Pasteels 2010), in contrast to variable glandular sources responsible for the pheromones release (Quennedey et al. 2008; Bordereau and Pasteels 2010; Sillam-Dussès 2010, 2011).

In addition to the identical nature of the trail-following pheromone in Stylotermitidae and Kalotermitidae, these families also share similar way-of-life, feeding on a single piece of wood and unable to colonise additional pieces of wood (Abe 1987; Liang et al. 2017; Buček et al. 2022). This is also the case for the Stolotermitidae: *Stolotermes*, Archotermopsidae, Rhinotermitidae: *Termitogeton*, and Serritermitidae. In contrast, many other termites (*Mastotermes*, all Hodotermitidae, Stolotermitidae: *Porotermes*, Kalotermitidae: *Paraneotermes*, most of Rhinotermitidae and Termitidae) reveal ability to forage outside their nests through a network of subterranean galleries or trails covered by faecal sheetings (Abe 1987; Bordereau and Pasteels 2010). It is easy to understand that having a powerful trail-following pheromone is essential for workers living in a complex system of galleries and orienting themselves between the foraging territory and the nest. However, the need of such a pheromone in termites living confined to a single wood item for the whole colony duration is less obvious. Even though *Stylotermes* colonies live in trunks serving as a nesting and foraging site, the trail-following pheromone may be needed to recruit nestmates for defensive purposes, as already hypothesized for Kalotermitidae (Sillam-Dussès et al. 2009a).

DOE appears to be the only compound acting as a trail-following pheromone of *Stylotermes* spp. On the other hand, several termite groups are known to rely upon minor pheromonal compounds, which may show a synergistic effect (Sillam-Dussès et al. 2009b, 2010) or maintain the species-specific the trails (Bordereau and Pasteels 2010). Our results show that DOE elicits trail-following activity comparable to sternal gland extracts, and the existence of minor compound is thus not needed to provoke a sensitive trail-following behaviour in *Stylotermes*. Moreover, the multi-component trail-following pheromones are known only from species foraging outside of the nest

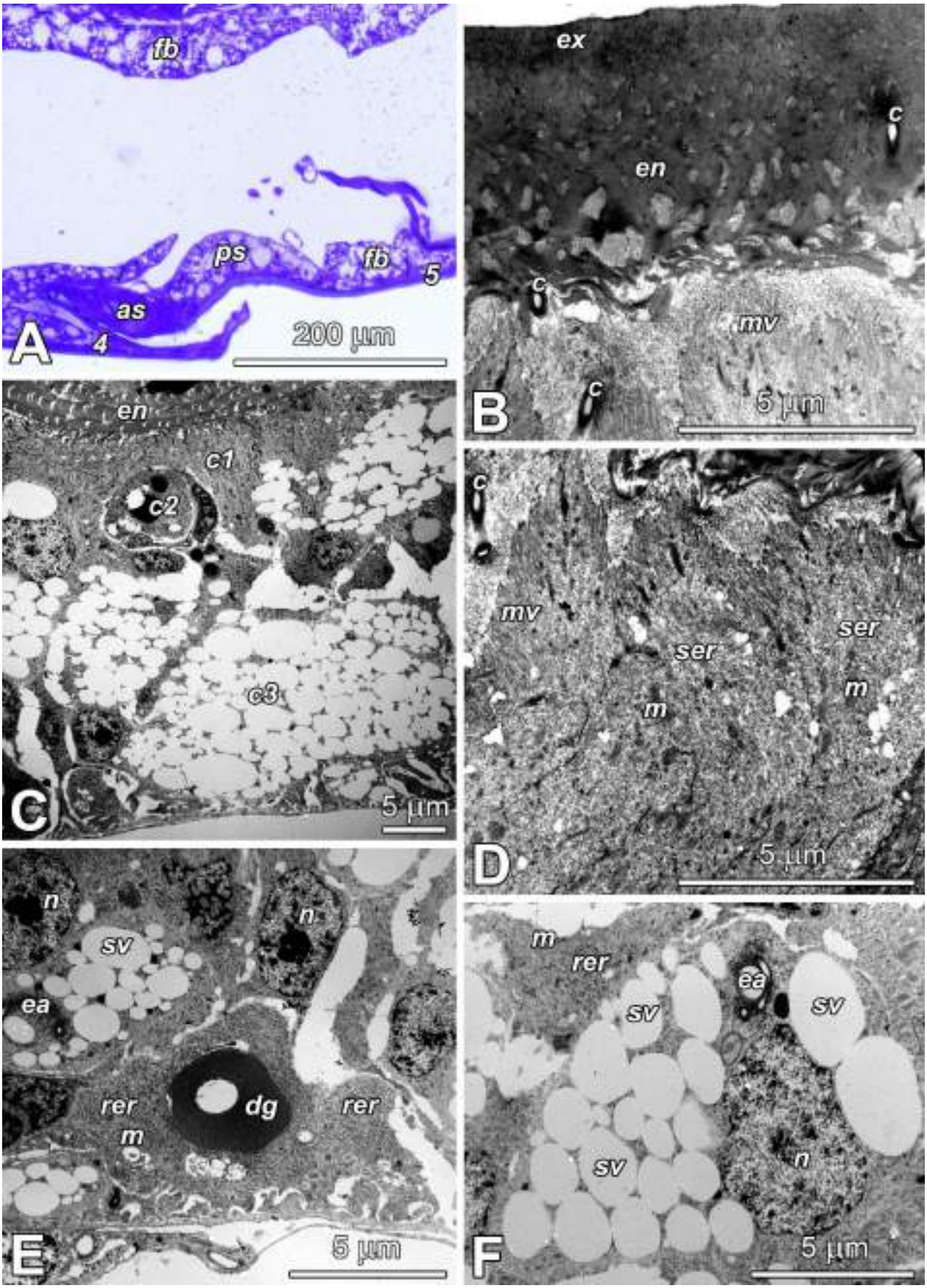
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Fig. 2 The structure of the sternal gland and the secretory cells in *Stylotermes faveolus* worker. **(A)** Sagittal section of the sternal gland. **(B)** The ultrastructure of the cuticle overlying the sternal gland. **(C)** The ultrastructure of the anterior part of the sternal gland. **(D)** The ultrastructure of the apical portion of the class I secretory cell. **(E)** The ultrastructure of the class II secretory cell. **(F)** The ultrastructure of the class III secretory cell. Abbreviations: 4, the fourth abdominal sternite; 5, the fifth abdominal sternite; as, anterior part of the sternal gland; c, canal of the class III secretory cell; c1, class I secretory cell; c2, class II secretory cell; c3, class III secretory cell; dg, electron-dense granule; ea, end apparatus; en, endocuticle; ex, exocuticle; fb, fat body; m, mitochondria; ps, posterior part of the sternal gland; rer, rough endoplasmic reticulum; ser, smooth endoplasmic reticulum; sv, secretory vesicle

(e.g. (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol, and neocembrene in *Prorhinotermes* (Rupf and Roisin 2008; Sillam-Dussès et al. 2005, 2009b) or in several *Nasutitermes* species (Sillam-Dussès et al. 2010), while species living confined to the same piece of wood always have a single pheromonal compound (e.g. (3*Z*)-dodec-3-en-1-ol in several *Cryptotermes* species (Sillam-Dussès et al. 2009a).

The nature of the trail-following pheromone confirms the close relationship between Stylotermitidae and Kalotermitidae, which has already been underlined by morphological and phylogenetic studies (Liang et al. 2017; Wu et al. 2018; Buček et al. 2019). The Stylotermitidae is clearly intermediate between the Kalotermitidae and the remaining families of the Neoisoptera clade to which the Stylotermitidae belongs (Holmgren and Holmgren 1917; Liang et al. 2017; Wu et al. 2018; Buček et al. 2019). With the present study, trail-following pheromones are identified in all termite families except the Hodotermitidae. Future studies

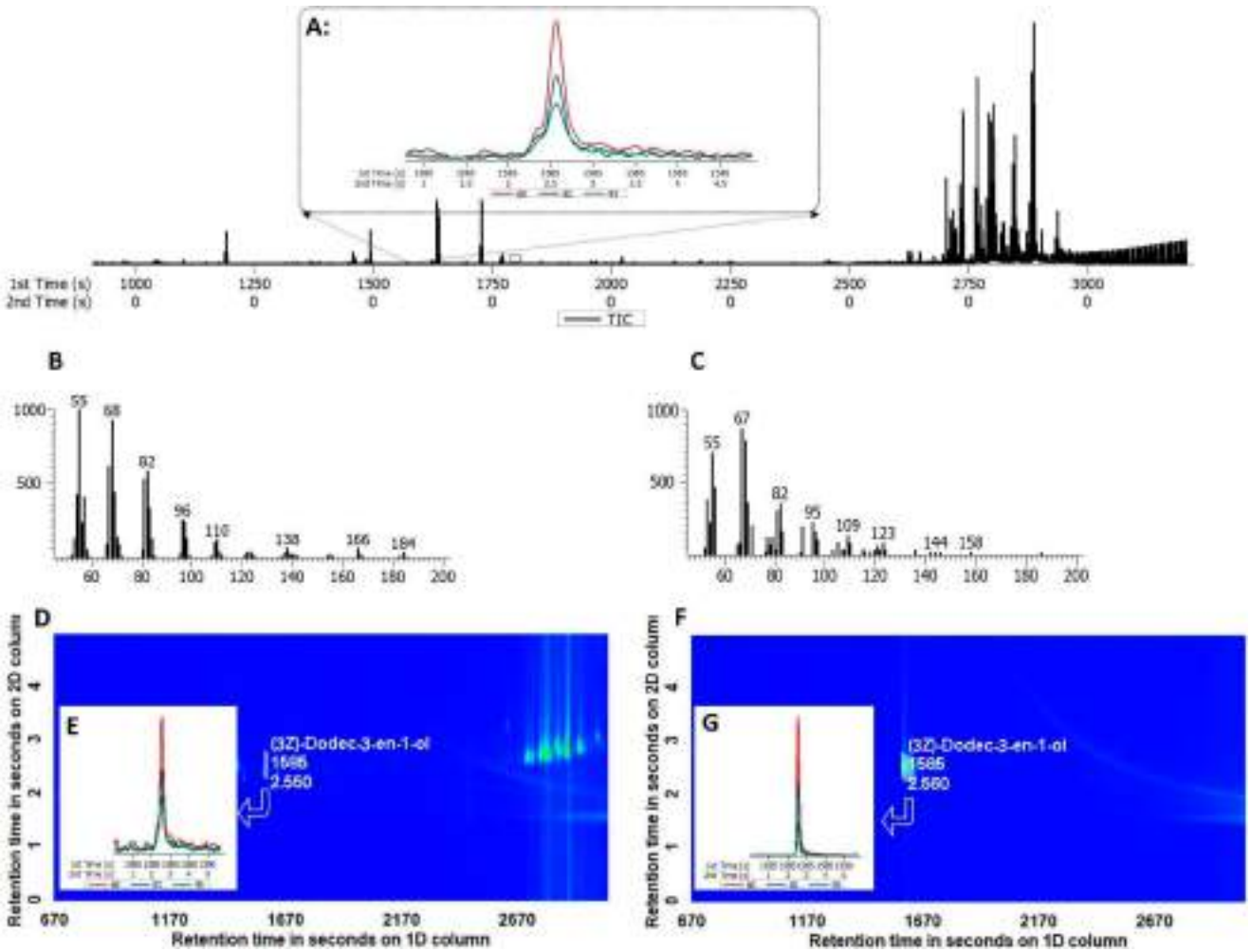

Fig. 3 Chemical identification of the trail-following pheromone of *Stylotermes faveolus*. **A**: Extracted ion chromatogram from sternal gland extracts, showing the specific masses of (*Z*)-dodec-3-en-1-ol (red - m/z 68, blue - m/z 82, green - m/z 95) on total ion chromatogram background (black); **B**: Mass spectrum of (*Z*)-dodec-3-en-1-ol from NIST mass spectral library; **C**: Corresponding deconvoluted mass spectrum in sample; **D**: Contour plot of 2D chromatogram from sternal gland extracts, mass m/z 68 is extracted; **E**: Zoom of 2D chromatogram of sternal gland extracts showing the specific masses of (*3Z*)-dodec-3-en-1-ol (DOE; red - m/z 68, blue - m/z 82, green - m/z 95); **F**: Contour plot of 2D chromatogram of DOE standard solution, mass m/z 68 is extracted; G: Zoom of 2D DOE standard solution chromatogram showing the specific masses (red - m/z 68, blue - m/z 82, green - m/z 95)

Table 1 Mean distances (cm ± SD) followed by *Stylotermes faveolus* workers (n = 30) or by *S. halumicus* workers (n = 30) on artificial trails. These trails were made with *S. faveolus* sternal gland extracts or (3*Z*)-dodec-3-en-1-ol (DOE) in trail-following bioassays. Trail-following with hexane (control) was 0 in all tests. The activity threshold was set to the minimum concentration that elicits termites to follow a mean distance of more than 3 cm (length of the Y stem, the maximum distance being 10 cm)

Tested substance	Mean length of trail followed (cm ± SD)	
	Stylotermes faveolus	*Stylotermes halumicus*
Sternal gland 10^{-1} eq/cm	7.7 ± 3.5 S	Not tested
DOE 10^{-4} ng/cm	2.5 ± 2.6 NS	3.1 ± 3.3 S
DOE 10^{-3} ng/cm	3.7 ± 3.2 S	9.9 ± 0.6 S
DOE 10^{-2} ng/cm	8.5 ± 2.5 S	9.9 ± 0.5 S
DOE 10^{-1} ng/cm	8.4 ± 2.9 S	10 ± 0 S
DOE 1 ng/cm	5.7 ± 3.7 S	6.3 ± 3.9 S

S = significant; NS = non-significant

Table 2 Choice made by *Stylotermes faveolus* workers (n = 30) between trails made of *S. faveolus* sternal gland extracts and trails made of (3*Z*)-dodec-3-en-1-ol at different concentrations in two-choice Y-open field trail-following bioassays. Data were evaluated using a Monte Carlo test based on χ^2 test at the level of $p < 0.05$

(3*Z*)-dodec-3-en-1-ol		Sternal gland of *Stylotermes faveolus* at 10^{-1} eq/cm	*n*	*p*
10^{-2} ng/cm	2	28	30	S***
10^{-1} ng/cm	18	12	30	NS

NS = non-significant

S*** = significant ($p < 0.001$)

should investigate this family comprising termite species with very peculiar features like functional compound eyes and unusual foraging behaviour in workers. The identification of the trail-following pheromone of species belonging to Hodotermitidae will allow us to get a full picture of the evolution of trail-following pheromones in termites.

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Data Availability Not applicable.

Declarations

Competing Interests Authors declare no competing interests.

Ethical Approval Not applicable.

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