TRAIL-FOLLOWING PHEROMONES IN THE TERMITE SUBFAMILY SYNTERMITINAE (BLATTODEA, TERMITOIDAE, TERMITIDAE)

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Running title: Trail-following pheromones in Syntermitinae

Abstract – Trail-following behaviour is a key to termite ecological success, allowing to orient themselves between the nesting and foraging sites. This behaviour is controlled by specific trail-following pheromones produced always by the abdominal sternal gland occurring in all termite species and developmental stages. Trail-following communication was studied in a broad spectrum of species, but the "higher" termites (i.e. Termitidae) from the subfamily Syntermitinae remain surprisingly neglected. To fill this gap, we studied the trail-following pheromone in 6 genera and 9 species of Syntermitinae. Our chemical and behavioural experiments showed that (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol is the single component of the pheromone of all the termite species studied, except *Silvestritermes euamignathus.* This species produces both (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol and neocembrene, but only (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol elicits trail-following behaviour. Our results indicate the importance of (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol, the most widespread communication compound in termites, but also the repeated switches to other common pheromones as exemplified by *S. euamignathus*.

Key Words – Termite, dodecatrienol, dodecadienol, neocembrene, Termitidae, Isoptera.

INTRODUCTION

Pheromones, and especially trail-following pheromones, play a key role for coordinating collective activities in termite societies. However, very little is known about the chemical nature of these trail-following pheromones, in comparison to other social insects such as ants (Bordereau and Pasteels 2011). The research in this field is hampered by the difficulty to extract and isolate the active compounds making up the pheromones. The most powerful technique in this field is the solid-phase micro-extraction (SPME) coupled to gas chromatography-mass spectrometry (GC-MS). SPME-GC-MS allows direct assessment of the sternal gland secretion, the only source of trail-following pheromones in termites (Noirot 1969; Quennedey et al. 2008). However, only 7 different compounds are known to play the role of trail-following pheromones in more than 60 termite species studied so far (Bordereau and Pasteels 2011; Gössinger 2019; Sillam-Dussès 2010, 2011). (*E*)-2,6,10-Trimethyl-5,9-undecadien-1-ol is known to be the trail-following pheromone in Mastotermitidae, and Stolotermitidae (Sillam-Dussès et al. 2007), and (*Z*)-dodec-3-en-1-ol in Kalotermitidae (Sillam-Dussès et al. 2009a). *Syn*-4,6-Dimethyldodecanal, *syn*-4,6-dimethylundecan-1-ol, and (10*Z*,13*Z*)-nonadeca-10,13-dien-2-one have been identified as the trail-following pheromone of *Zootermopsis* spp. (Archotermopsidae) (Bordereau et al. 2010), *Hodotermopsis sjoestedti* (Archotermopsidae) (Lacey et al. 2011), and *Glossotermes oculatus* (Serritermitidae) (Hanus et al. 2012), respectively. (3*Z*,6*Z*,8*E*)-Dodeca-3,6,8-trien-1-ol (dodecatrienol) is also known to be a major or a minor component of the trail-following pheromone in all Rhinotermitidae (Sillam-Dussès et al. 2006; Wobst et al. 1999). Surprisingly, the trail-following pheromone consisting of both neocembrene and dodecatrienol occurs in *Prorhinotermes simplex* (Rhinotermitidae) (Sillam-Dussès et al. 2005, 2009b), *Amitermes evuncifer* (Termitinae) (Anani Kotoklo et al. 2010), and many Nasutitermitinae (Sillam-Dussès et al. 2010). Particular Macrotermitinae species use (*Z*)-dodec-3-en-1-ol, (3*Z*,6*Z*)-dodeca-3,6-dien-1-ol (dodecadienol) or dodecatrienol as trail-following pheromones (Bordereau et al. 1993; Peppuy et al. 2001a, b; Robert et al. 2004; Wen et al. 2017), while all other Termitidae use dodecatrienol only (Sillam-Dussès et al. 2006) (Figure 1).

The low diversity of trail-following pheromones in termites, or so-called pheromonal parsimony, opened questions on the species-specificity of these pheromones. To understand this phenomenon, the knowledge on the trail nature in major termite lineages is needed, but

only some groups were studied in this respect while others received no attention so far. One of such group is the Neotropical mandibulate nasutes or subfamily Syntermitinae (Termitidae). Even the phylogenetic position of this group has been long debated, due to striking morphological similarities to the subfamily Nasutitermitinae (Ahmad 1950; Allee et al. 1949; Donovan et al. 2000; Eggleton 2001; Noirot 2001; Ohkuma et al. 2004). The subfamily Syntermitinae was erected by Engel & Krishna (2004), and recent phylogenies confirmed its monophyly and deep separation from Nasutitermitinae (Bourguignon et al. 2015, 2017; Buček et al. 2019; Inward et al. 2007; Rocha et al. 2012, 2017).

The absence of data on the trail-following pheromone within Syntermitinae stimulated our interest into this particular taxon comprising 99 species with wood- or soil-feeding habit (Krishna et al. 2013). Here, we report on the identity of the trail-following pheromone in *Syntermes grandis* and eight more Syntermitinae species.

METHODS AND MATERIALS

Insects. Table 1 shows the studied species and the localities where they were collected.

Gland extracts. Dissections were made in the native country of the termite species, i.e. Brazil or French Guiana. Sternal glands were carefully dissected on the fifth abdominal sternite from cold anesthetized workers under a stereomicroscope with microscissors, extracted with bidistilled hexane for 6 hours, and then diluted for bioassays. All extracts were stored at -20°C before use. Extracts were used at concentration 0.1 gland equivalent per 1 µl of the extract in hexane.

Chemical Analyses. Termites were maintained in a climate-controlled room (26°C, 60% RH) in France and chemical analyses were performed on termites a few days after they were delivered to France. The principle consisted in comparing by gas chromatography-mass spectrometry (GC-MS) the compounds isolated using solid-phase micro-extraction (SPME) collections from the worker's sternal gland opening and the surface of the non-glandular integument as a control. This approach allowed us to identify the compounds specific to the sternal gland secretion. According to the size of termites and the activity of the sternal gland, collections from 10 to 100 workers were used for a GC-MS analysis. The fibre was desorbed in

the injection port of a gas chromatograph for 3 min for gas chromatography (GC) and GC-MS analyses. GC and GC-MS analyses were carried out with a 5973N Mass Selective Detector coupled to a 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) fitted with a split-splitless injector and a DB™-Wax column (30 m×0.32 mm ID, 0.5 µm film thickness, J&W Scientific, Folsom, CA, USA) or an Equity 5 column (30 m×0.32 mm ID, 0.25 µm film thickness, Supelco). Columns were heated from 40 to 240°C at 5°C min−1. Helium was used as carrier gas at a constant velocity of 37 cm/s. The temperature of the injector was set to 250°C. The column was interfaced directly to the ion source of the mass spectrometer through a heated transfer line maintained at 250°C. Electron-ionization (EI) mass spectra were obtained at 70 eV with the instrument scanning from m/z 29 to 450, and the source maintained at 230°C (for more details, see Sillam-Dussès et al. 2007). Once identified, the quantity of pheromone was estimated from a rough comparison of the GC peaks of the standards and of the pheromone detected by rubbing the termites or by injecting in sternal gland extracts.

Standards. Synthetic dodecatrienol was kindly provided by the Nitto Denko Japanese Company (purity 99%). Dodecadienol was synthesized by F. D. Boyer (purity 99%) (see details in Robert et al. 2004). Neocembrene was purified from tergal glands of alate females of *Nasutitermes voeltzkowi* (purity degree 98%) (see details in Sillam-Dussès et al. 2005).

Bioassays. They were performed in the native country of the termite species, i.e. Brazil or French Guiana (Table 2). Artificial trails made with sternal gland extracts or standards were assayed using a Y open-field bioassay on Whatman N°1 filter paper discs (15 cm in diameter) with a 120° angle between each branch. On the Y stem (3 cm) and on one of the Y branches (7 cm), a trail was drawn with a microlitre 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 base of the Y and the other Y branch. One termite was placed inside a release chamber made of a small plastic vial (55 mm in diameter) with the 2-5 mm wide opening (according to the species size) located at the base of the Y. The distance traveled by each worker on the trail was measured. The activity threshold was arbitrarily fixed as the minimum concentration inducing termites to travel a mean distance of more than 3 cm, the maximal response being 10 cm. For every test, a new individual and a new filter paper were used to prevent any effects from behavioural conditioning or trail reinforcement. The arms of the trail were randomly

149 interchanged between replicates to prevent any bias. 30 workers were tested for each
150 concentration and for each species in all bioassays. All bioassays were performed under
151 standardized conditions (25±1°C, red dimmed light). For a choice test, the number of termites
152 that chose a trail or another was recorded, and the data were compared using χ^2 test (S* =
153 $p<0.05$, NS = non significant). When several species were available at the same time, such
154 choice tests were also performed to test species-specificity, i.e. one worker has the choice
155 between a trail made with the sternal gland extract of his own species and a trail made with
156 the sternal gland extract of another species. The number of workers tested was between 14
157 and 29.
158
159 RESULTS
160 *Syntermitinae Trail-following Pheromones Exemplified on* Syntermes grandis. The SPME-GC-
161 MS profiles of the worker sternal gland showed the presence of common cuticular
162 hydrocarbons (C_{25} to C_{30}), and only one peak specific to the glandular surface compared to the
163 control (Figure 2). The comparison with the synthetic standard proved the identity of the peak
164 as dodecatrienol. Trail-following bioassays showed a high activity of this alcohol in eliciting
165 trail-following, with a threshold at 10^{-4} ng/cm of trail, an optimal activity at 10^{-2} ng/cm and a
166 decreasing activity from 10^{-1} ng/cm (Table 2).
167
168 *Other Syntermitinae*. Comparable results were obtained in *Cornitermes bequaerti*, *C.*
169 *cumulans*, *C. snyderi*, *Cyrilliotermes angulariceps*, *Labiotermes labralis*, and *Embiratermes*
170 *neotenicus* in which dodecatrienol was always detected (Table 3).
171 Bioassays showed a very high activity of dodecatrienol in eliciting trail-following in all
172 *Cornitermes* spp., *Cyrilliotermes angulariceps*, and *L. labralis* (Table 2). *E. neotenicus* showed
173 even much higher sensitivity with an activity threshold at 10^{-6} ng/cm and an optimal activity
174 at 10^{-4} ng/cm.
175 *Silvestritermes euamignathus* differed from the other studied Syntermitinae by producing two
176 specific components, dodecadienol and neocembrene (Table 3). Dodecadienol was active in
177 eliciting trail-following from 10^{-2} ng/cm, whereas neocembrene did not elicit trail-following at
178 any tested concentration. Various mixtures of dodecadienol and neocembrene did not
179 improve trail-following activity (0.8±0.3 cm with a mixture of dodecadienol at 10^{-3} ng/cm and
180 neocembrene at 10^{-1} ng/cm; 8.7±0.9 cm with a mixture of dodecadienol at 10^{-1} ng/cm and

neocembrene at 1 ng/cm). Unfortunately, the limited availability of the biological material did not allow us to test other mixtures of dodecadienol and neocembrene. Moreover, workers of *S. euamignathus* were able to follow trails of dodecatrienol but they were 10 times less sensitive to dodecatrienol than to dodecadienol. Neocembrene activity was tested in three Syntermitinae representatives, without eliciting any trail-following activity (for details see Table 2).

Species-specificity of Trail-following Pheromones. No species-specificity of trail-following could be observed with our experimental conditions between *Cornitermes* and *Syntermes* (Tables 4 and 5). Workers of *C. cumulans* even preferentially followed the trails made of sternal gland extracts of workers of *C. bequaerti*, in which dodecatrienol was perhaps present at a slightly higher concentration.

DISCUSSION

All Syntermitinae species studied except *Silvestritermes euamignathus* secreted a trail-following pheromone comprized very likely only of dodecatrienol, with a very low activity threshold, similarly to other species using the same trail-following pheromone (Bordereau and Pasteels 2011; Sillam-Dussès 2010, 2011). The scent trails are of monocomponent composition in most of studied Syntermitinae, and no species-specificity was observed using our standardiszed design, but we cannot completely exclude that the trail-following pheromone contained also some minor compounds that could not be detected.

The sensitivity to dodecatrienol varies by about 3 orders of magnitude, with *Embiratermes neotenicus* workers responding to 10^{-6} ng/cm, whereas others like *Cornitermes snyderi* and *Labiotermes labralis* workers did not respond until concentration 10^{-3} ng/cm. Such sensitivity difference has already been observed in other termite species which have the same trail-following pheromone (e.g. in several species belonging to Kalotermitidae (Sillam-Dussès et al. 2009a) or to Nasutitermitinae (Sillam-Dussès et al. 2010)). It is likely that this sensitivity difference is due to some inherent biological reason. However, because of the limited information available, we cannot say if it is linked to the size of the sternal gland (see Quennedey et al. 2008) containing a quantity more or less important of the pheromone according to the species. The size difference between *Syntermes grandis* and the other termite

species studied is sometimes very important, *S. grandis* being among the biggest termite species in the world. When the concentration of the pheromone is too high, the antennal receptors get probably saturated and thus the workers cannot follow the artificial trails easily. The lack of trail specificity caused by a single-component trail-following pheromone was already observed in Mastotermitidae and Stolotermitidae (using (*E*)-2,6,10-trimethyl-5,9-undecadien-1-ol), various Kalotermitidae (using (*Z*)-dodec-3-en-1-ol), various Rhinotermitidae (using dodecatrienol), or Macrotermitinae (using dodecatrienol), with preferences explained only by the quantity of the pheromone (Bordereau et al. 1993; Sillam-Dussès et al. 2007, 2009a; Wobst et al. 1999). Syntermitinae species are of "separate" life type (*sensu* Abe 1987) or "central-site nesters" (*sensu* Shellman-Reeve 1997), and thus the food is collected in the foraging area and transported to the nest. Although one would expect a selective pressure on direct discrimination by species-specific trail-following pheromones due to similar niche/food realized by many Syntermitinae species (grass-feeding in *Syntermes* and *Cornitermes*, humus/soil-feeding in all others), the observed pattern is much easier. The reason may lie in a very short longevity of the scent trails counted in units of hours (Bordereau and Pasteels 2011), i.e. the time when using a trail by another species is highly unlikely. When these rare encounters take place, the species- and colony-specific recognition is allowed by distinct profiles of the cuticular hydrocarbons (Howard and Blomquist 1982, 2005).

Silvestritermes euamignathus significantly differs from all other Syntermitinae. Not only did this species secrete two compounds specific to the sternal gland surface, dodecadienol and neocembrene, but workers only followed dodecadienol. Furthermore, a mixture of the two components did not enhance trail-following, so the role of neocembrene remains unknown. It might be possible that it is used as species-specific signal, but unfortunately, this termite species was available in very low number allowing us only to detect neocembrene, but not to perform species-specificity bioassays. Neocembrene is a common component of trail-following pheromones, and occurs together with dodecatrienol as a functional compound in a number of species. It is a dominant component in many Nasutitermitinae (Sillam-Dussès et al. 2010), minor component in *Prorhinotermes simplex* (Rhinotermitidae) (Sillam-Dussès et al. 2009b), and the ratios of the two components are not known in *Amitermes evuncifer* (Termitinae) (Kotoklo et al. 2010). Neocembrene in *S. euamignathus* might actually repel sympatric termite species. Chemically mediated conflict-avoidance strategy has been observed in the termite host *Constrictotermes* and its inquiline

Inquilinitermes, whose spatial separation is based on recognition cues (present in the whole body washes) but not in the trail-following pheromone (extracted from the dissected sternal glands) (Cristaldo et al. 2014; Jirošová et al. 2016). *S. euamignathus* is the fourth example of neocembrene production by the sternal gland in termites. Although the most plausible explanation due to phylogenetic position of respective taxa (see Bourguignon et al. 2015, 2017) is thus four independent acquisitions of neocembrene synthesis, the apparent lack of function of the compound in *S. euamignathus* shows the need to search for this compound in other termite species, as it might be more widespread and perhaps plays different functions in particular taxa. Interestingly, dodecadienol as a trail-following pheromone occurs only in *S. euamignathus* and few Macrotermitinae (Robert et al. 2004; Wen et al. 2014, 2017). Another interesting observation is that *S. euamignathus* workers were sensitive to dodecatrienol, although they do not biosynthesize it, as it has been previously found for some other termite species with a different trail-following pheromone (Bordereau and Pasteels 2011; Matsumura et al. 1972).

Chemical evolution of trail-following pheromones is impressively conservative in termites. Dodecatrienol, the most common component of the trail-following pheromones, represents additional apomorphy of advanced termites grouped in Neoisoptera, as it was already discovered in most of Rhinotermitidae and Termitidae. To confirm this hypothesis, the identification of the trail-following pheromone of *Stylotermes* seems essential, as it represents a sister group to all remaining Neoisoptera (Buček et al. 2019; Wu et al. 2018). Even more interesting is a high chemical parsimony observed in termites, i.e. use of the same compound in different context for different purposes. All compounds discussed here, dodecatrienol, dodecadienol and neocembrene, may act as both, sex and trail-following pheromone, although not always in the same species (for review see Bordereau and Pasteels 2011 or Sillam-Dussès 2011). Dodecatrienol has been identified as the sex pheromone of some Syntermitinae species, such as *Embiratermes neotenicus* (Dolejšová et al. 2018) and *Cornitermes* spp. (Bordereau et al. 2011), or *Prorhinotermes simplex* (Rhinotermitidae; Hanus et al. 2009). Dodecadienol is the sex pheromone of *Silvestritermes* spp. (Dolejšová et al. 2018), and neocembrene is the sex pheromone of *Nasutitermes* spp. (Bordereau and Pasteels 2011).

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AUTHOR CONTRIBUTIONS

The study was designed by DSD, AR and CB. Material preparation, data collection and analysis were performed by DSD, JŠ, TB, PW, ES, EMC, CL and CB. The first draft of the manuscript was written by DSD, JŠ and CB and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

The authors declare that they have no conflict of interest.

REFERENCES

Abe T (1987) Evolution of life types in termites. In: Kawano S, Connell JH, Hikada T (eds) Evolution and coadaptation in biotic communities, Tokyo, Tokyo University press, pp 125–148.

Allee WC, Emerson AE, Park O, Park T, Schmidt KP (1949) Principles of animal ecology. W. B. Saunders company, Philadelphia and London.

Ahmad A (1950) Phylogeny of termite genera. Bull Am Mus Nat Hist 95(2):43–86.

Kotoklo EA, Sillam-Dussès D, Ketoh G, Sémon É, Robert A, Bordereau C, Glitho I (2010) Identification of the trail-following pheromone of the pest termite *Amitermes evuncifer* (Isoptera: Termitidae). Sociobiol 55:1–10.

309 Bordereau C, Robert A, Laduguie N, Bonnard O, Le Quéré JL, Yamaoka R (1993) Détection du
310 (*Z*,*Z*,*E*)-3,6,8-dodecatrien-1-ol par les ouvriers et les essaimants de deux espèces de termites
311 champignonnistes : *Pseudacanthotermes spiniger* et *P. militaris* (Termitidae,
312 Macrotermitinae). Actes des Colloques Insectes Soc 8:145–149.
313 Bordereau C, Lacey MJ, Sémon É, Braekman JC, Ghostin J, Robert A, Shellman Sherman J,
314 Sillam-Dussès D (2010) Sex pheromones and trail-following pheromone in the basal termites
315 *Zootermopsis nevadensis* (Hagen) and *Z. angusticollis* (Hagen) (Isoptera, Termopsidae,
316 Termopsinae). Biol J Linn Soc 100:519–530.
317 Bordereau C, Cancello EM, Sillam-Dussès D, Sémon E (2011) Sex-pairing pheromones and
318 reproductive isolation in three sympatric *Cornitermes* species (Isoptera, Termitidae,
319 Syntermitinae). J Insect Physiol 57: 469–474.
320 Bordereau C, Pasteels JM (2011) Pheromones and chemical ecology of dispersal and foraging
321 in termites. In: Bignell DE, Roisin Y, Lo N (eds) Biology of termites, a modern synthesis.
322 Springer, pp 279–320.
323 Bourguignon T, Lo N, Cameron SL, Šobotník J, Hayashi Y, Shigenobu S, Watanabe D, Roisin Y,
324 Miura T, Evans TA (2015) The evolutionary history of termites as inferred from 66
325 mitochondrial genomes. Mol Biol Evol. 32:406–421.
326 Bourguignon T, Lo N, Šobotník J, Ho SYW, Iqbal N, Coissac E, Lee M, Jendryka M, Sillam-Dussès
327 D, Křížková B, Roisin Y, Evans TA (2017) Mitochondrial phylogenomics resolves the global
328 spread of higher termites, ecosystem engineers of the tropics. Mol Biol Evol 34:589–597.
329 Buček A, Šobotník J, He S, Shi M, McMahon DP, Holmes EC, Roisin Y, Lo N, Bourguignon T
330 (2019) Evolution of termite symbiosis informed by transcriptome-based phylogenies. Curr Biol
331 24(21):3728–3734.e4.
332 Cristaldo PF, DeSouza O, Krasulová J, Jirošová A, Kutalová K, Rodrigues Lima E, Šobotník J,
333 Sillam-Dussès D (2014) Mutual use of trail-following chemical cues by a termite host and its
334 inquilines. PLoS ONE 9(1):e85315.
335 Dolejšová K, Křivánek J, Kalinová B, Hadravová R, Kyjaková P, Hanus R (2018) Sex-pairing
336 pheromones in three sympatric neotropical termite species (Termitidae: Syntermitinae). J
337 Chem Ecol 44:534–546.
338 Donovan SE, Jones DT, Sands WA, Eggleton P (2000) Morphological phylogenetics of termites
339 (Isoptera). Biol J Linn Soc 70:467–513.

340 Eggleton P (2001) Termites and trees: a review of recent advances in termite phylogenetics.
341 Insectes Soc 48:187–193.
342 Engel MS, Krishna K (2004) Family-group names for termites (Isoptera). Am Museum Novitates
343 3432: 1–9.
344 Gössinger E (2019) Chemistry of the secondary metabolites of termites. In: Kinghorn AD, Falk
345 H, Gibbons S, Kobayashi J, Asakawa Y, Liu J-K (Eds) Progress in the Chemistry of Organic Natural
346 Products, Vol. 109. Springer, Cham, Switzerland, pp 1-384.
347 Hanus R, Šobotník J, Krasulová J, Jiroš P, Žáček P, Kalinová B, Dolejšová K, Cvačka J,
348 Bourguignon T, Roisin Y, Lacey MJ, Sillam-Dussès D (2012) Nonadecadienone, a new termite
349 trail-following pheromone identified in *Glossotermes oculatus* (Serritermitidae). Chem Senses
350 37:55–63.
351 Howard RW, Blomquist GJ (1982) Chemical Ecology and Biochemistry of Insect Hydrocarbons.
352 Ann Rev Entomol 27:149–172.
353 Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect
354 hydrocarbons. Ann Rev Entomol 50:371–393.
355 Inward DJG, Vogler AP, Eggleton P (2007) A comprehensive phylogenetic analysis of termites
356 (Isoptera) illuminates key aspects of their evolutionary biology. Mol Phylogenet Evol 44:953–
357 967.
358 Jirošová A, Sillam-Dussès D, Kyjaková P, Kalinová B, Dolejšová K, Jančařík A, Majer P, Cristaldo
359 PF, Hanus R (2016) Smells like home: chemically mediated co-habitation of two termite
360 species in a single nest. J Chem Ecol 42(10):1070–1081.
361 Lacey MJ, Sémon É, Krasulová J, Sillam-Dussès D, Robert A, Cornette R, Hoskovec M, Žáček P,
362 Valterová I, Bordereau C (2011) Chemical communication in termites: *syn*-4,6-
363 dimethylundecan-1-ol as trail-following pheromone, *syn*-4,6-dimethylundecanal and (5*E*)-
364 2,6,10-trimethylundeca-5,9-dienal as the respective male and female sex pheromones in
365 *Hodotermopsis sjoestedti* (Isoptera, Archotermopsidae). J Insect Physiol 57:1585–1591.
366 Matsumura F, Jewett DM, Coppel HC (1972) Interspecific response of termite to synthetic trail-
367 following substances. J Econ Entomol 65:600–602.
368 Noirot C (1969) Glands and secretions. In: Krishna K, Weesner FM (eds) Biology of termites,
369 Vol. I. New York, Academic Press, pp 89-123.
370 Noirot C (2001) The gut of termites (Isoptera), comparative anatomy, systematics, phylogeny.
371 II.- Higher termites (Termitidae). Ann Soc Entomol Fr 37:431–471.

372 Ohkuma M, Yuzawa H, Amornsak W, Sornnuwat Y, Takematsu Y, Yamada A, Vongkaluang C,
373 Sarnthoy O, Kirtibutr N, Noparatnaraporn N, Kudo T, Inoue T (2004) Molecular phylogeny of
374 Asian termites (Isoptera) of the families Termitidae and Rhinotermitidae based on
375 mitochondrial COII sequences. Mol Phylogenetics Evol 31:701–710.
376 Peppuy A, Robert A, Sémon E, Giniès C, Letteré M, Bonnard O, Bordereau C (2001a) (*Z*)-Dodec-
377 3-en-1-ol, a novel termite trail pheromone identified after solid phase microextraction from
378 *Macrotermes annandalei*. J Insect Physiol 47:445–453.
379 Peppuy A, Robert A, Sémon E, Bonard O, Son NT, Bordereau C (2001b) Species-specificity of
380 trail pheromones of fungus-growing termites from northern Vietnam. Insectes Soc 48:245–
381 250.
382 Quennedey A, Sillam-Dussès D, Robert A, Bordereau C (2008) The fine structural organization
383 of sternal glands of pseudergates and workers in termites (Isoptera): A comparative survey.
384 Arthropod Struct Dev 37:168–185.
385 Robert A, Peppuy A, Sémon E, Boyer FD, Lacey MJ, Bordereau C (2004) A new C12 alcohol
386 identified as a sex pheromone and a trail-following pheromone in termites: the diene (*Z*,*Z*)-
387 dodeca-3,6-dien-1ol. Naturwiss 91:34–39.
388 Rocha MM, Cancello EM, Carrijo TF (2012) Neotropical termites: revision of *Armitermes*
389 Wasmann (Isoptera, Termitidae, Syntermitinae) and phylogeny of the Syntermitinae. Syst
390 Entomol 37:793–827.
391 Rocha MM, Morales-Corrêa e Castro AC, Cuezzo C, Cancello EM (2017) Phylogenetic
392 reconstruction of Syntermitinae (Isoptera, Termitidae) based on morphological and molecular
393 data. PLoS ONE 12(3):e0174366.
394 Shellman-Reeve JS (1997) The spectrum of eusociality in termites. In: Choe JC, Crespi
395 BJ (Eds) The evolution of social behavior in insects and arachnids. Cambridge University Press,
396 New York, pp 52-93.
397 Sillam-Dussès D (2010) Trail pheromones and sex pheromones in termites. Hauppauge, NY:
398 Novinka, Nova Science Publishers. 79 pp.
399 Sillam-Dussès D (2011) Trail pheromones and sex pheromones in termites: glandular origin,
400 chemical nature, and potential use in pest management. In: Gregory IM (Ed) Pheromones:
401 Theories, Types and Uses. New York, New York.

Sillam-Dussès D, Sémon E, Moreau C, Valterová I, Šobotník J, Robert A, Bordereau C (2005) Neocembrene A, a major component of the trail-following pheromone in the genus *Prorhinotermes* (Insecta, Isoptera, Rhinotermitidae). Chemoecol 15:1–6.

Sillam-Dussès D, Robert A, Sémon E, Lacey M, Bordereau C (2006) Trail-following pheromones and phylogeny in termites. Proceedings of the XV Congress of IUSSI, Washington, DC, pp 100–101.

Sillam-Dussès D, Sémon E, Lacey MJ, Robert A, Lenz M, Bordereau C (2007) Trail-following pheromones in basal termites, with special reference to *Mastotermes darwiniensis*. J Chem Ecol 33:1960–1977.

Sillam-Dussès D, Sémon E, Robert A, Bordereau C (2009a) (*Z*)-Dodec-3-en-1-ol, a common major component of the trail-following pheromone in the termites Kalotermitidae. Chemoecol 19(2):103–108.

Sillam-Dussès D, Kalinová B, Jiroš P, Březinová A, Cvačka J, Hanus R, Šobotník J, Bordereau C, Valterová I (2009b) Identification by GC-EAD of the two-component trail-following pheromone of *Prorhinotermes simplex* (Isoptera, Rhinotermitidae, Prorhinotermitinae). J Insect Physiol 55:751–757.

Sillam-Dussès D, Sémon E, Robert A, Cancello E, Lenz M, Valterová, I, Bordereau C (2010) Identification of multi-component trail pheromones in the most evolutionarily derived termites, the Nasutitermitinae (Termitidae). Biol J Linnean Soc 99:20–27.

Wen P, Ji B-Z, Sillam-Dussès D (2014) Trail communication is regulated by two trail pheromone components in the fungus-growing termite *Odontotermes formosanus* Shiraki. PLoS ONE 9(3):e90906.

Wen X, Wen P, Dahlsjö CAL, Šobotník J, Sillam-Dussès D (2017) Breaking the cipher: ant eavesdropping on the variational trail pheromone of its termite prey. Proc R Soc London 284:20170121.

Wobst B, Farine JP, Giniès C, Sémon E, Robert A, Bonnard O, Connétable S, Bordereau C (1999) (3*Z*,6*Z*,8*E*)-3,6,8-Dodecatrien-1-ol, a major component of trail-following pheromone in two sympatric termite species *Reticulitermes lucifugus grassei* and *R. santonensis*. J Chem Ecol 25:1305–1318.

Wu LW, Bourguignon T, Šobotník J, Wen P, Liang WR, Li HF (2018) Phylogenetic position of the enigmatic termite family Stylotermitidae. Invertebr Syst 32:1111–1117.

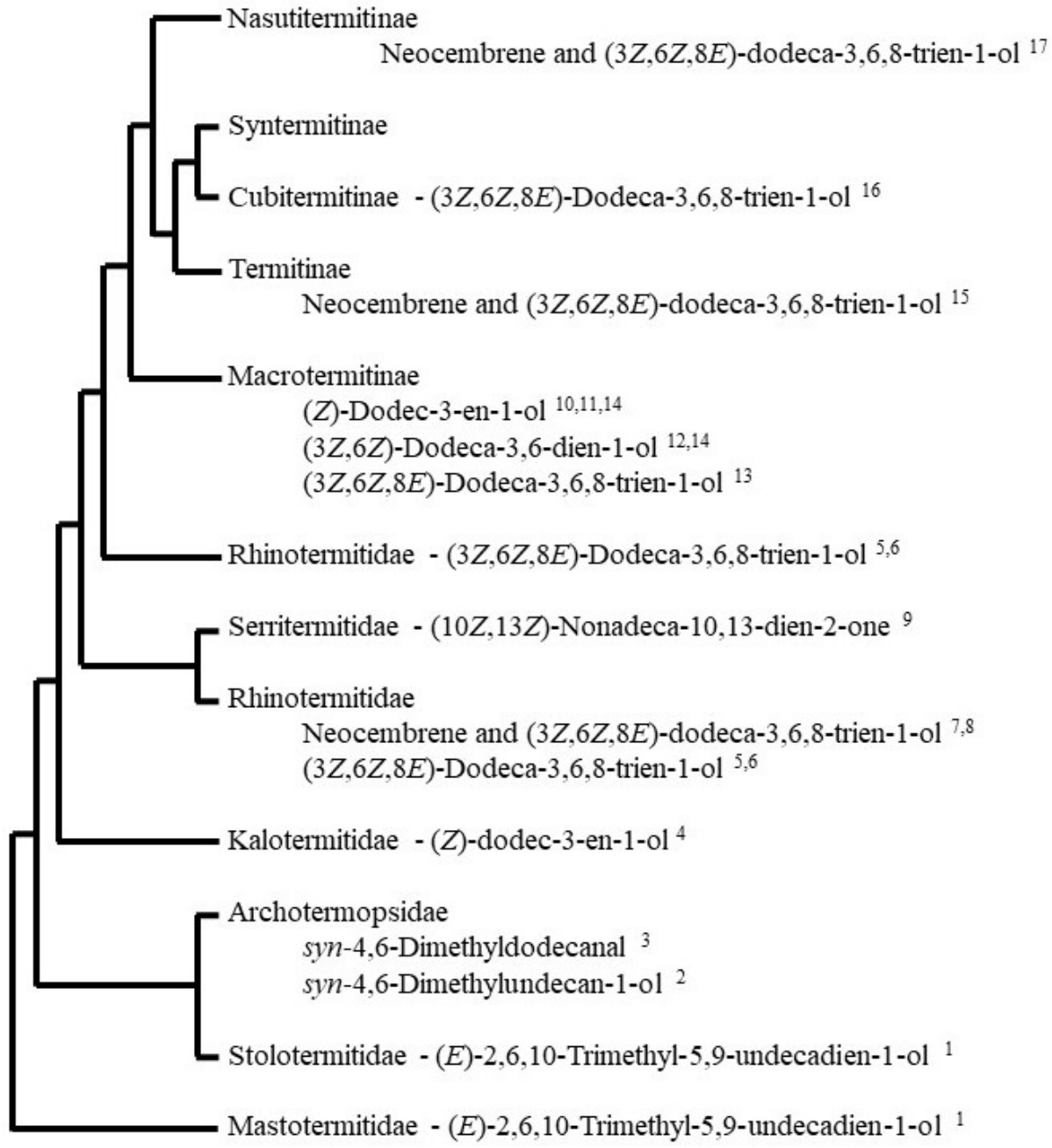

Fig. 1 SIMPLIFIED PHYLOGENY OF THE MAIN TERMITE FAMILIES AND SUB-FAMILIES (ACCORDING TO BOURGUIGNON ET AL. 2015) WITH THE CHEMICAL NATURE OF THE TRAIL-FOLLOWING PHEROMONES IDENTIFIED IN AT LEAST ONE SPECIES BELONGING TO THESE FAMILIES OR SUB-FAMILIES. References : [1]Sillam-Dussès et al. 2007; [2]Lacey et al. 2011; [3]Bordereau et al. 2010; [4]Sillam-Dussès et al. 2009a; [5]Sillam-Dussès et al. 2006; [6]Wobst et al. 1999; [7]Sillam-Dussès et al. 2005; [8]Sillam-Dussès et al. 2009b; [9]Hanus et al. 2012; [10]Peppuy et al. 2001a; [11]Peppuy et al. 2001b; [12]Robert et al. 2004; [13]Bordereau et al. 1993; [14]Wen et al. 2014; [15]Anani Kotoklo et al. 2010; [16]Sillam-Dussès et al. 2006; [17]Sillam-Dussès et al. 2010

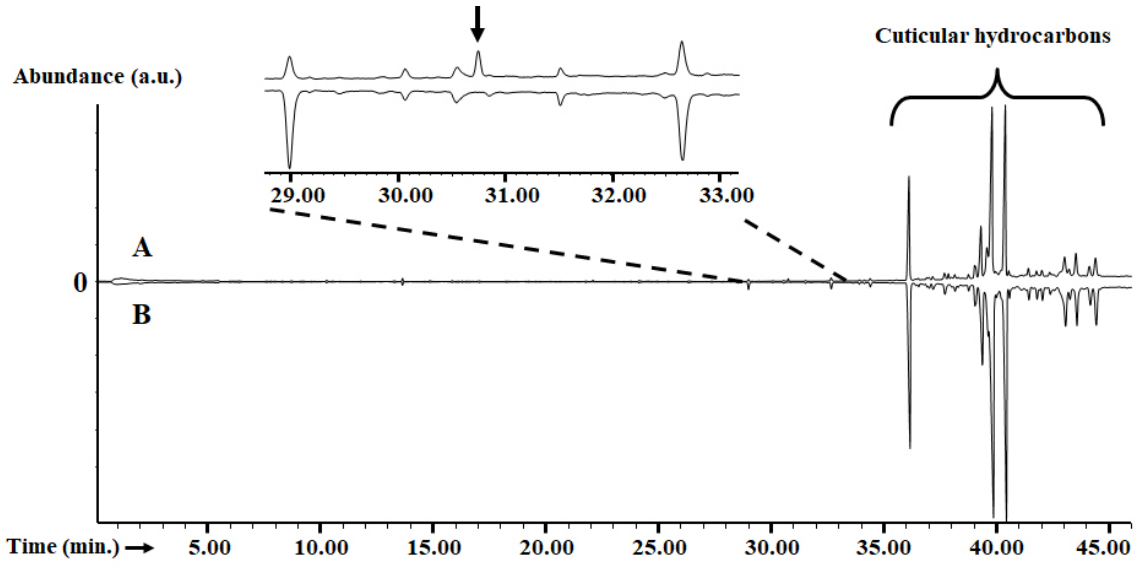

Fig. 2 GC PROFILES OF SPME COLLECTIONS OF THE STERNAL GLAND SURFACE (A) AND THE ABDOMINAL TERGAL SURFACE (B) OF *Syntermes grandis* WORKERS. Peaks show the same compounds common to both surfaces and correspond to cuticular hydrocarbons (C25-C30) except for one peak specific to the sternal gland surface (arrow). This compound was identified as (3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol

451 LEGENDS OF TABLES

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453 Table 1 LIST OF STUDIED SPECIES WITH THE PLACE OF COLLECTION

Species	Place of collection
Cornitermes bequaerti Emerson, 1952	Area of Botucatu, State of São Paulo, Brazil
Cornitermes cumulans (Kollar, 1832)	Area of Botucatu, State of São Paulo, Brazil
Cornitermes snyderi Emerson, 1952	Area of Botucatu, State of São Paulo, Brazil
Cyrilliotermes angulariceps (Mathews, 1977)	Area of Petit Saut, French Guiana
Embiratermes neotenicus (Holmgren, 1906)	Area of Petit Saut, French Guiana
Labiotermes labralis (Holmgren, 1906)	Area of Petit Saut, French Guiana
Silvestritermes euhamignathus (Silvestri, 1901)	Area of Brasília, Brazil
Syntermes grandis (Rambur, 1842)	Area of Botucatu, State of São Paulo, Brazil

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457 Table 2 TRAIL-FOLLOWING BIOASSAYS WITH 10 CM-LONG ARTIFICIAL TRAILS MADE OF
458 SYNTHETIC (3*Z*,6*Z*,8*E*)-DODECA-3,6,8-TRIEN-1OL, (3*Z*,6*Z*)-DODECA-3,6-DIEN-1-OL, OR
459 NEOCEMBRENE

Tested species	Concentration (ng/cm)							
	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10
	(3*Z*,6*Z*,8*E*)-Dodeca-3,6,8-trien-1-ol							
Cornitermes bequaerti	-	1.8±0.7	6.6±0.8	7.5±0.7	9.5±0.3	10	4.9±0.8	0.8±0.4
Cornitermes cumulans	-	2.4±0.5	3.7±0.5	7.8±0.5	9.5±0.3	9.3±0.3	3.9±0.6	1.2±0.4
Cornitermes snyderi	-	-	1.3±0.2	8.1±0.8	10	8.7±0.9	3.2±0.9	-
Cyrilliotermes angulariceps	-	0.2±0.4	5.9±3.8	7.6±3.3	5.8±3.7	6.5±3.9	2.3±2.6	-
Embiratermes neotenicus	6.7±3.9	8.2±3.3	8.9±2.5	7.7±3.8	6.4±4.0	4.3±3.8	3.6±3.4	-
Labiotermes labralis	-	-	1.9±1.5	4.9±3.4	7.9±2.8	9.3±1.8	8.9±2.0	-
Silvestritermes euamignathus	-	-	-	1.1±0.2	1.7±0.5	7.7±1.0	5.3±1.1	-
Syntermes grandis	-	-	4.7±1.2	7.3±1.1	9.6±0.4	5.9±1.3	4.9±1.3	-
	(3*Z*,6*Z*)-Dodeca-3,6-dien-1-ol							
Silvestritermes euamignathus	-	-	0.3±0.1	1.5±0.3	7.9±0.5	10	9.7±0.3	5.2±0.8
	Neocembrene							
Embiratermes neotenicus	-	-	-	-	0.5±0.8	0.3±0.5	0.6±1.0	-
Labiotermes labralis	-	-	-	0.3±0.7	0.5±1.1	0.1±0.3	0.8±1.2	-
Silvestritermes euamignathus	-	-	0.3±0.1	0.4±0.1	0.2±0.1	0.3±0.1	0.3±0.1	0.3±0.1

460 Values are distances of open-field trail-following (mean±SD in cm, n=30). Hexane, used as a
461 control, was never followed

462 Table 3 DETECTION OF (3*Z*,6*Z*,8*E*)-DODECA-3,6,8-TRIEN-1OL, (3*Z*,6*Z*)-DODECA-3,6-DIEN-1-OL,
463 OR NEOCEMBRENE BY GC-MS AFTER SPME IN THE TESTED SYNTERMITINAE SPECIES

Tested species	Detection of chemical compounds (ng/worker)		
	(3*Z*,6*Z*,8*E*)-dodeca-3,6,8-trien-1-ol	(3*Z*,6*Z*)-dodeca-3,6-dien-1-ol	Neocembrene
Cornitermes bequaerti	+	-	-
Cornitermes cumulans	+	-	-
Cornitermes snyderi	+	-	-
Cyrilliotermes angulariceps	+	-	-
Embiratermes neotenicus	+	-	-
Labiotermes labralis	+	-	-
Silvestritermes euamignathus	-	+	+
Syntermes grandis	+	-	-

464 The techniques used do not allow a reliable quantification of the compounds, so only the
465 presence (+) or the absence (-) of the compounds is indicated

Table 4 CHOICE TRAIL-FOLLOWING BIOASSAYS BETWEEN *Cornitermes bequarti* AND *C. cumulans*

Tested species	Number of termites following trails made of sternal gland extracts of		n	χ^2
	C. bequaerti	*C. cumulans*		
Cornitermes bequaerti	15	9	24	NS
Cornitermes cumulans	20	9	29	S*

All trails were made of worker sternal gland extracts at 10^{-1} gland/cm (χ^2 test, S* = $p<0.05$, NS = non significant)

Table 5 CHOICE TRAIL-FOLLOWING BIOASSAYS BETWEEN *Cornitermes cumulans* AND *Syntermes grandis*

Tested species	Number of termites following trails made of sternal gland extracts of		n	χ^2
	C. cumulans	*S. grandis*		
Cornitermes cumulans	7	7	14	NS
Syntermes grandis	6	9	15	NS

All trails were made of worker sternal gland extracts at 10^{-1} gland/cm (χ^2 test, NS = non significant)