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## Trail-Following Pheromones in the Termite Subfamily Syntermitinae (Blattodea, Termitoidea, Termitidae)

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1 TRAIL-FOLLOWING PHEROMONES IN THE TERMITE SUBFAMILY SYNTERMITINAE  
2 (BLATTODEA, TERMITOIDEA, TERMITIDAE)

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32 Running title: Trail-following pheromones in Syntermitinae

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35 **Abstract** – Trail-following behaviour is a key to termite ecological success, allowing to orient  
36 themselves between the nesting and foraging sites. This behaviour is controlled by specific  
37 trail-following pheromones produced always by the abdominal sternal gland occurring in all  
38 termite species and developmental stages. Trail-following communication was studied in a  
39 broad spectrum of species, but the “higher” termites (i.e. Termitidae) from the subfamily  
40 Syntermitinae remain surprisingly neglected. To fill this gap, we studied the trail-following  
41 pheromone in 6 genera and 9 species of Syntermitinae. Our chemical and behavioural  
42 experiments showed that (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol is the single component of the  
43 pheromone of all the termite species studied, except *Silvestritermes euamignathus*. This  
44 species produces both (3Z,6Z)-dodeca-3,6-dien-1-ol and neocembrene, but only (3Z,6Z)-  
45 dodeca-3,6-dien-1-ol elicits trail-following behaviour. Our results indicate the importance of  
46 (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol, the most widespread communication compound in  
47 termites, but also the repeated switches to other common pheromones as exemplified by *S.*  
48 *euamignathus*.

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

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## INTRODUCTION

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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

85 only some groups were studied in this respect while others received no attention so far. One  
86 of such group is the Neotropical mandibulate nasutes or subfamily Syntermitinae  
87 (Termitidae). Even the phylogenetic position of this group has been long debated, due to  
88 striking morphological similarities to the subfamily Nasutitermitinae (Ahmad 1950; Allee et al.  
89 1949; Donovan et al. 2000; Eggleton 2001; Noirot 2001; Ohkuma et al. 2004). The subfamily  
90 Syntermitinae was erected by Engel & Krishna (2004), and recent phylogenies confirmed its  
91 monophyly and deep separation from Nasutitermitinae (Bourguignon et al. 2015, 2017; Buček  
92 et al. 2019; Inward et al. 2007; Rocha et al. 2012, 2017).

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

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#### METHODS AND MATERIALS

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

101

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

108

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

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

130

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

135

136 *Bioassays.* They were performed in the native country of the termite species, i.e. Brazil or  
137 French Guiana (Table 2). Artificial trails made with sternal gland extracts or standards were  
138 assayed using a Y open-field bioassay on Whatman N°1 filter paper discs (15 cm in diameter)  
139 with a 120° angle between each branch. On the Y stem (3 cm) and on one of the Y branches  
140 (7 cm), a trail was drawn with a microlitre syringe containing 1 µl of extract per 1 cm of trail.  
141 Another extract or hexane as a control was deposited in the same conditions on the base of  
142 the Y and the other Y branch. One termite was placed inside a release chamber made of a  
143 small plastic vial (55 mm in diameter) with the 2-5 mm wide opening (according to the species  
144 size) located at the base of the Y. The distance traveled by each worker on the trail was  
145 measured. The activity threshold was arbitrarily fixed as the minimum concentration inducing  
146 termites to travel a mean distance of more than 3 cm, the maximal response being 10 cm. For  
147 every test, a new individual and a new filter paper were used to prevent any effects from  
148 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 ( $25\pm 1^\circ\text{C}$ , 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  $\chi^2$  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.

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## 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 ( $\text{C}_{25}$  to  $\text{C}_{30}$ ), 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*, *Cyrelliatermes angulariceps*, *Labiatermes 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., *Cyrelliatermes 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 ( $0.8\pm 0.3$  cm with a mixture of dodecadienol at  $10^{-3}$  ng/cm and  
180 neocembrene at  $10^{-1}$  ng/cm;  $8.7\pm 0.9$  cm with a mixture of dodecadienol at  $10^{-1}$  ng/cm and

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

187  
188 *Species-specificity of Trail-following Pheromones.* No species-specificity of trail-following  
189 could be observed with our experimental conditions between *Cornitermes* and *Syntermes*  
190 (Tables 4 and 5). Workers of *C. cumulans* even preferentially followed the trails made of  
191 sternal gland extracts of workers of *C. bequaerti*, in which dodecatrienol was perhaps present  
192 at a slightly higher concentration.

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## DISCUSSION

195

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

203 The sensitivity to dodecatrienol varies by about 3 orders of magnitude, with *Embiratermes*  
204 *neotenicus* workers responding to  $10^{-6}$  ng/cm, whereas others like *Cornitermes snyderi* and  
205 *Labiotermes labralis* workers did not respond until concentration  $10^{-3}$  ng/cm. Such sensitivity  
206 difference has already been observed in other termite species which have the same trail-  
207 following pheromone (e.g. in several species belonging to Kalotermitidae (Sillam-Dussès et al.  
208 2009a) or to Nasutitermitinae (Sillam-Dussès et al. 2010)). It is likely that this sensitivity  
209 difference is due to some inherent biological reason. However, because of the limited  
210 information available, we cannot say if it is linked to the size of the sternal gland (see  
211 Quennedey et al. 2008) containing a quantity more or less important of the pheromone  
212 according to the species. The size difference between *Syntermes grandis* and the other termite



213 species studied is sometimes very important, *S. grandis* being among the biggest termite  
214 species in the world. When the concentration of the pheromone is too high, the antennal  
215 receptors get probably saturated and thus the workers cannot follow the artificial trails easily.  
216 The lack of trail specificity caused by a single-component trail-following pheromone was  
217 already observed in Mastotermitidae and Stolotermitidae (using (*E*)-2,6,10-trimethyl-5,9-  
218 undecadien-1-ol), various Kalotermitidae (using (*Z*)-dodec-3-en-1-ol), various Rhinotermitidae  
219 (using dodecatrienol), or Macrotermitinae (using dodecatrienol), with preferences explained  
220 only by the quantity of the pheromone (Bordereau et al. 1993; Sillam-Dussès et al. 2007,  
221 2009a; Wobst et al. 1999). Syntermitinae species are of “separate” life type (*sensu* Abe 1987)  
222 or “central-site nesters” (*sensu* Shellman-Reeve 1997), and thus the food is collected in the  
223 foraging area and transported to the nest. Although one would expect a selective pressure on  
224 direct discrimination by species-specific trail-following pheromones due to similar niche/food  
225 realized by many Syntermitinae species (grass-feeding in *Syntermes* and *Cornitermes*,  
226 humus/soil-feeding in all others), the observed pattern is much easier. The reason may lie in  
227 a very short longevity of the scent trails counted in units of hours (Bordereau and Pasteels  
228 2011), i.e. the time when using a trail by another species is highly unlikely. When these rare  
229 encounters take place, the species- and colony-specific recognition is allowed by distinct  
230 profiles of the cuticular hydrocarbons (Howard and Blomquist 1982, 2005).

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

245 *Inquilinitermes*, whose spatial separation is based on recognition cues (present in the whole  
246 body washes) but not in the trail-following pheromone (extracted from the dissected sternal  
247 glands) (Cristaldo et al. 2014; Jirošová et al. 2016). *S. euamignathus* is the fourth example of  
248 neocembrene production by the sternal gland in termites. Although the most plausible  
249 explanation due to phylogenetic position of respective taxa (see Bourguignon et al. 2015,  
250 2017) is thus four independent acquisitions of neocembrene synthesis, the apparent lack of  
251 function of the compound in *S. euamignathus* shows the need to search for this compound in  
252 other termite species, as it might be more widespread and perhaps plays different functions  
253 in particular taxa. Interestingly, dodecadienol as a trail-following pheromone occurs only in *S.*  
254 *euamignathus* and few Macrotermitinae (Robert et al. 2004; Wen et al. 2014, 2017). Another  
255 interesting observation is that *S. euamignathus* workers were sensitive to dodecatrienol,  
256 although they do not biosynthesize it, as it has been previously found for some other termite  
257 species with a different trail-following pheromone (Bordereau and Pasteels 2011; Matsumura  
258 et al. 1972).

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

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#### AUTHOR CONTRIBUTIONS

290

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

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296 The authors declare that they have no conflict of interest.

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#### REFERENCES

300

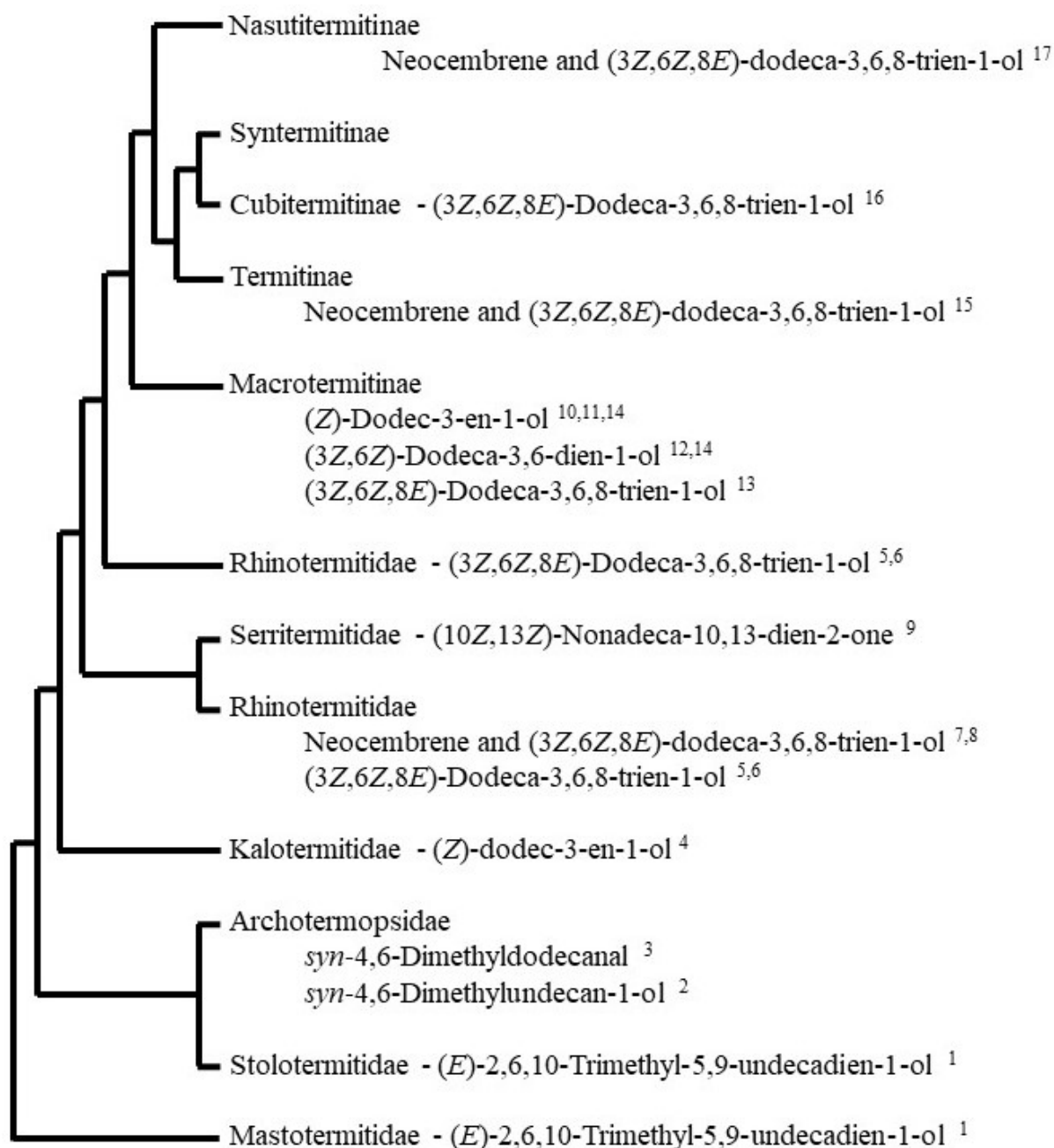
- 301 Abe T (1987) Evolution of life types in termites. In: Kawano S, Connell JH, Hikada T (eds)  
302 Evolution and coadaptation in biotic communities, Tokyo, Tokyo University press, pp 125–148.
- 303 Allee WC, Emerson AE, Park O, Park T, Schmidt KP (1949) Principles of animal ecology. W. B.  
304 Saunders company, Philadelphia and London.
- 305 Ahmad A (1950) Phylogeny of termite genera. Bull Am Mus Nat Hist 95(2):43–86.
- 306 Kotoklo EA, Sillam-Dussès D, Ketoh G, Sémon É, Robert A, Bordereau C, Glitho I (2010)  
307 Identification of the trail-following pheromone of the pest termite *Amitermes evuncifer*  
308 (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, Canello 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, Canello 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, Cuezco C, Canello 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.

- 402 Sillam-Dussès D, Sémon E, Moreau C, Valterová I, Šobotník J, Robert A, Bordereau C (2005)  
403 Neocembrene A, a major component of the trail-following pheromone in the genus  
404 *Prorhinotermes* (Insecta, Isoptera, Rhinotermitidae). Chemoecol 15:1–6.
- 405 Sillam-Dussès D, Robert A, Sémon E, Lacey M, Bordereau C (2006) Trail-following pheromones  
406 and phylogeny in termites. Proceedings of the XV Congress of IUSI, Washington, DC, pp 100–  
407 101.
- 408 Sillam-Dussès D, Sémon E, Lacey MJ, Robert A, Lenz M, Bordereau C (2007) Trail-following  
409 pheromones in basal termites, with special reference to *Mastotermes darwiniensis*. J Chem  
410 Ecol 33:1960–1977.
- 411 Sillam-Dussès D, Sémon E, Robert A, Bordereau C (2009a) (Z)-Dodec-3-en-1-ol, a common  
412 major component of the trail-following pheromone in the termites Kalotermitidae.  
413 Chemoecol 19(2):103–108.
- 414 Sillam-Dussès D, Kalinová B, Jiroš P, Březinová A, Cvačka J, Hanus R, Šobotník J, Bordereau C,  
415 Valterová I (2009b) Identification by GC-EAD of the two-component trail-following pheromone  
416 of *Prorhinotermes simplex* (Isoptera, Rhinotermitidae, Prorhinotermitinae). J Insect Physiol  
417 55:751–757.
- 418 Sillam-Dussès D, Sémon E, Robert A, Canello E, Lenz M, Valterová, I, Bordereau C (2010)  
419 Identification of multi-component trail pheromones in the most evolutionarily derived  
420 termites, the Nasutitermitinae (Termitidae). Biol J Linnean Soc 99:20–27.
- 421 Wen P, Ji B-Z, Sillam-Dussès D (2014) Trail communication is regulated by two trail pheromone  
422 components in the fungus-growing termite *Odontotermes formosanus* Shiraki. PLoS ONE  
423 9(3):e90906.
- 424 Wen X, Wen P, Dahlsjö CAL, Šobotník J, Sillam-Dussès D (2017) Breaking the cipher: ant  
425 eavesdropping on the variational trail pheromone of its termite prey. Proc R Soc London  
426 284:20170121.
- 427 Wobst B, Farine JP, Giniès C, Sémon E, Robert A, Bonnard O, Connétable S, Bordereau C (1999)  
428 (3Z,6Z,8E)-3,6,8-Dodecatrien-1-ol, a major component of trail-following pheromone in two  
429 sympatric termite species *Reticulitermes lucifugus grassei* and *R. santonensis*. J Chem Ecol  
430 25:1305–1318.
- 431 Wu LW, Bourguignon T, Šobotník J, Wen P, Liang WR, Li HF (2018) Phylogenetic position of  
432 the enigmatic termite family Stylotermitidae. Invertebr Syst 32:1111–1117.



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434 **Fig. 1** SIMPLIFIED PHYLOGENY OF THE MAIN TERMITE FAMILIES AND SUB-FAMILIES

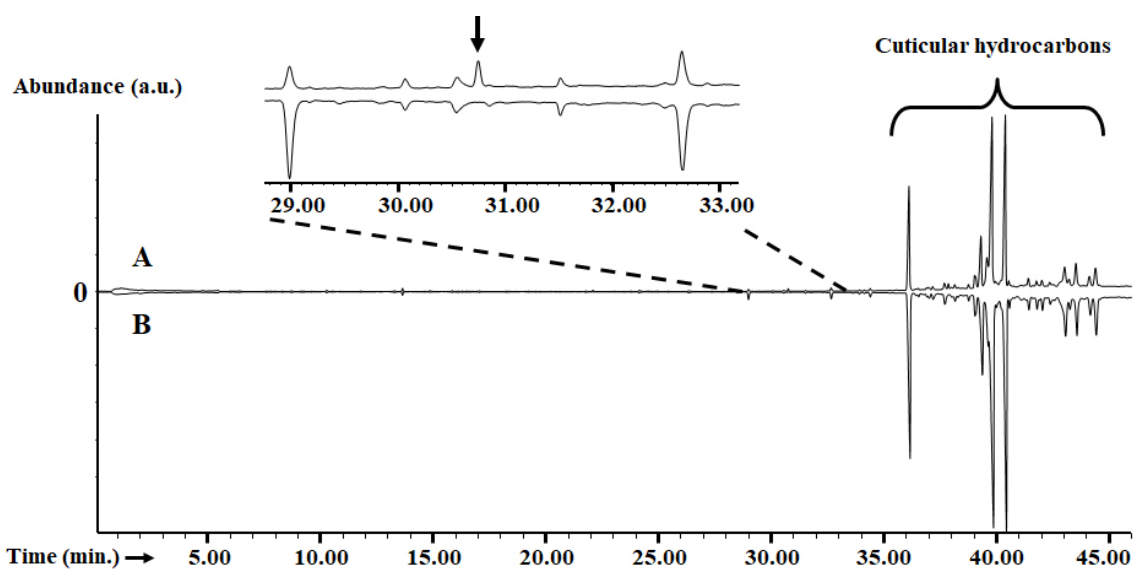
435 (ACCORDING TO BOURGUIGNON ET AL. 2015) WITH THE CHEMICAL NATURE OF THE TRAIL-

436 FOLLOWING PHEROMONES IDENTIFIED IN AT LEAST ONE SPECIES BELONGING TO THESE

437 FAMILIES OR SUB-FAMILIES. References : <sup>1</sup>Sillam-Dussès et al. 2007; <sup>2</sup>Lacey et al. 2011;438 <sup>3</sup>Bordereau et al. 2010; <sup>4</sup>Sillam-Dussès et al. 2009a; <sup>5</sup>Sillam-Dussès et al. 2006; <sup>6</sup>Wobst et al.439 1999; <sup>7</sup>Sillam-Dussès et al. 2005; <sup>8</sup>Sillam-Dussès et al. 2009b; <sup>9</sup>Hanus et al. 2012; <sup>10</sup>Peppuy et440 al. 2001a; <sup>11</sup>Peppuy et al. 2001b; <sup>12</sup>Robert et al. 2004; <sup>13</sup>Bordereau et al. 1993; <sup>14</sup>Wen et al.441 2014; <sup>15</sup>Anani Kotoklo et al. 2010; <sup>16</sup>Sillam-Dussès et al. 2006; <sup>17</sup>Sillam-Dussès et al. 2010

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

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## LEGENDS OF TABLES

452

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

Species	Place of collection
<i>Cornitermes bequaerti</i> Emerson, 1952	Area of Botucatu, State of São Paulo, Brazil
<i>Cornitermes cumulans</i> (Kollar, 1832)	Area of Botucatu, State of São Paulo, Brazil
<i>Cornitermes snyderi</i> Emerson, 1952	Area of Botucatu, State of São Paulo, Brazil
<i>Cyrtillitermes angulariceps</i> (Mathews, 1977)	Area of Petit Saut, French Guiana
<i>Embiratermes neotenicus</i> (Holmgren, 1906)	Area of Petit Saut, French Guiana
<i>Labiatermes labralis</i> (Holmgren, 1906)	Area of Petit Saut, French Guiana
<i>Silvestritermes euhamignathus</i> (Silvestri, 1901)	Area of Brasília, Brazil
<i>Syntermes grandis</i> (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 (3Z,6Z,8E)-DODECA-3,6,8-TRIEN-1-OL, (3Z,6Z)-DODECA-3,6-DIEN-1-OL, OR  
 459 NEOCEMBRENE

Tested species	Concentration (ng/cm)							
	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>	10 <sup>-2</sup>	10 <sup>-1</sup>	1	10
	(3Z,6Z,8E)-Dodeca-3,6,8-trien-1-ol							
<i>Cornitermes bequaerti</i>	-	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
<i>Cornitermes cumulans</i>	-	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
<i>Cornitermes snyderi</i>	-	-	1.3±0.2	8.1±0.8	10	8.7±0.9	3.2±0.9	-
<i>Cyrelliatermes angulariceps</i>	-	0.2±0.4	5.9±3.8	7.6±3.3	5.8±3.7	6.5±3.9	2.3±2.6	-
<i>Embiratermes neotenicus</i>	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	-
<i>Labiatermes labralis</i>	-	-	1.9±1.5	4.9±3.4	7.9±2.8	9.3±1.8	8.9±2.0	-
<i>Silvestritermes euamignathus</i>	-	-	-	1.1±0.2	1.7±0.5	7.7±1.0	5.3±1.1	-
<i>Syntermes grandis</i>	-	-	4.7±1.2	7.3±1.1	9.6±0.4	5.9±1.3	4.9±1.3	-
	(3Z,6Z)-Dodeca-3,6-dien-1-ol							
<i>Silvestritermes euamignathus</i>	-	-	0.3±0.1	1.5±0.3	7.9±0.5	10	9.7±0.3	5.2±0.8
	Neocembrene							
<i>Embiratermes neotenicus</i>	-	-	-	-	0.5±0.8	0.3±0.5	0.6±1.0	-
<i>Labiatermes labralis</i>	-	-	-	0.3±0.7	0.5±1.1	0.1±0.3	0.8±1.2	-
<i>Silvestritermes euamignathus</i>	-	-	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 (3Z,6Z,8E)-DODECA-3,6,8-TRIEN-1OL, (3Z,6Z)-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)		
	(3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol	(3Z,6Z)-dodeca-3,6-dien-1-ol	Neocembrene
<i>Cornitermes bequaerti</i>	+	-	-
<i>Cornitermes cumulans</i>	+	-	-
<i>Cornitermes snyderi</i>	+	-	-
<i>Cyrlillitermes angulariceps</i>	+	-	-
<i>Embiratermes neotenicus</i>	+	-	-
<i>Labiatermes labralis</i>	+	-	-
<i>Silvestritermes euamignathus</i>	-	+	+
<i>Syntermes grandis</i>	+	-	-

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

466 Table 4 CHOICE TRAIL-FOLLOWING BIOASSAYS BETWEEN *Cornitermes bequaerti* AND *C.*  
 467 *cumulans*

Tested species	Number of termites following trails		n	$\chi^2$
	made of sternal gland extracts of			
	<i>C. bequaerti</i>	<i>C. cumulans</i>		
<i>Cornitermes bequaerti</i>	15	9	24	NS
<i>Cornitermes cumulans</i>	20	9	29	S*

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

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

Tested species	Number of termites following trails		n	$\chi^2$
	made of sternal gland extracts of			
	<i>C. cumulans</i>	<i>S. grandis</i>		
<i>Cornitermes cumulans</i>	7	7	14	NS
<i>Syntermes grandis</i>	6	9	15	NS

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

474