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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*.

49

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

51

52

INTRODUCTION

53
54
55 Pheromones, and especially trail-following pheromones, play a key role for coordinating
56 collective activities in termite societies. However, very little is known about the chemical
57 nature of these trail-following pheromones, in comparison to other social insects such as ants
58 (Bordereau and Pasteels 2011). The research in this field is hampered by the difficulty to
59 extract and isolate the active compounds making up the pheromones. The most powerful
60 technique in this field is the solid-phase micro-extraction (SPME) coupled to gas
61 chromatography-mass spectrometry (GC-MS). SPME-GC-MS allows direct assessment of the
62 sternal gland secretion, the only source of trail-following pheromones in termites (Noirot
63 1969; Quennedey et al. 2008). However, only 7 different compounds are known to play the
64 role of trail-following pheromones in more than 60 termite species studied so far (Bordereau
65 and Pasteels 2011; Gössinger 2019; Sillam-Dussès 2010, 2011).
66 (*E*)-2,6,10-Trimethyl-5,9-undecadien-1-ol is known to be the trail-following pheromone in
67 Mastotermitidae, and Stolotermitidae (Sillam-Dussès et al. 2007), and (*Z*)-dodec-3-en-1-ol in
68 Kalotermitidae (Sillam-Dussès et al. 2009a). *Syn*-4,6-Dimethyldodecanal, *syn*-4,6-
69 dimethylundecan-1-ol, and (10*Z*,13*Z*)-nonadeca-10,13-dien-2-one have been identified as the
70 trail-following pheromone of *Zootermopsis* spp. (Archotermopsidae) (Bordereau et al. 2010),
71 *Hodotermopsis sjoestedti* (Archotermopsidae) (Lacey et al. 2011), and *Glossotermes oculatus*
72 (Serritermitidae) (Hanus et al. 2012), respectively. (3*Z*,6*Z*,8*E*)-Dodeca-3,6,8-trien-1-ol
73 (dodecatrienol) is also known to be a major or a minor component of the trail-following
74 pheromone in all Rhinotermitidae (Sillam-Dussès et al. 2006; Wobst et al. 1999). Surprisingly,
75 the trail-following pheromone consisting of both neocembrene and dodecatrienol occurs in
76 *Prorhinotermes simplex* (Rhinotermitidae) (Sillam-Dussès et al. 2005, 2009b), *Amitermes*
77 *evuncifer* (Termitinae) (Anani Kotoklo et al. 2010), and many Nasutitermitinae (Sillam-Dussès
78 et al. 2010). Particular Macrotermitinae species use (*Z*)-dodec-3-en-1-ol, (3*Z*,6*Z*)-dodeca-3,6-
79 dien-1-ol (dodecadienol) or dodecatrienol as trail-following pheromones (Bordereau et al.
80 1993; Peppuy et al. 2001a, b; Robert et al. 2004; Wen et al. 2017), while all other Termitidae
81 use dodecatrienol only (Sillam-Dussès et al. 2006) (Figure 1).
82 The low diversity of trail-following pheromones in termites, or so-called pheromonal
83 parsimony, opened questions on the species-specificity of these pheromones. To understand
84 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.

97

98

METHODS AND MATERIALS

99

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⁻¹. 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 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*, *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 ± 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

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.

193

194

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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288

289

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.

295

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

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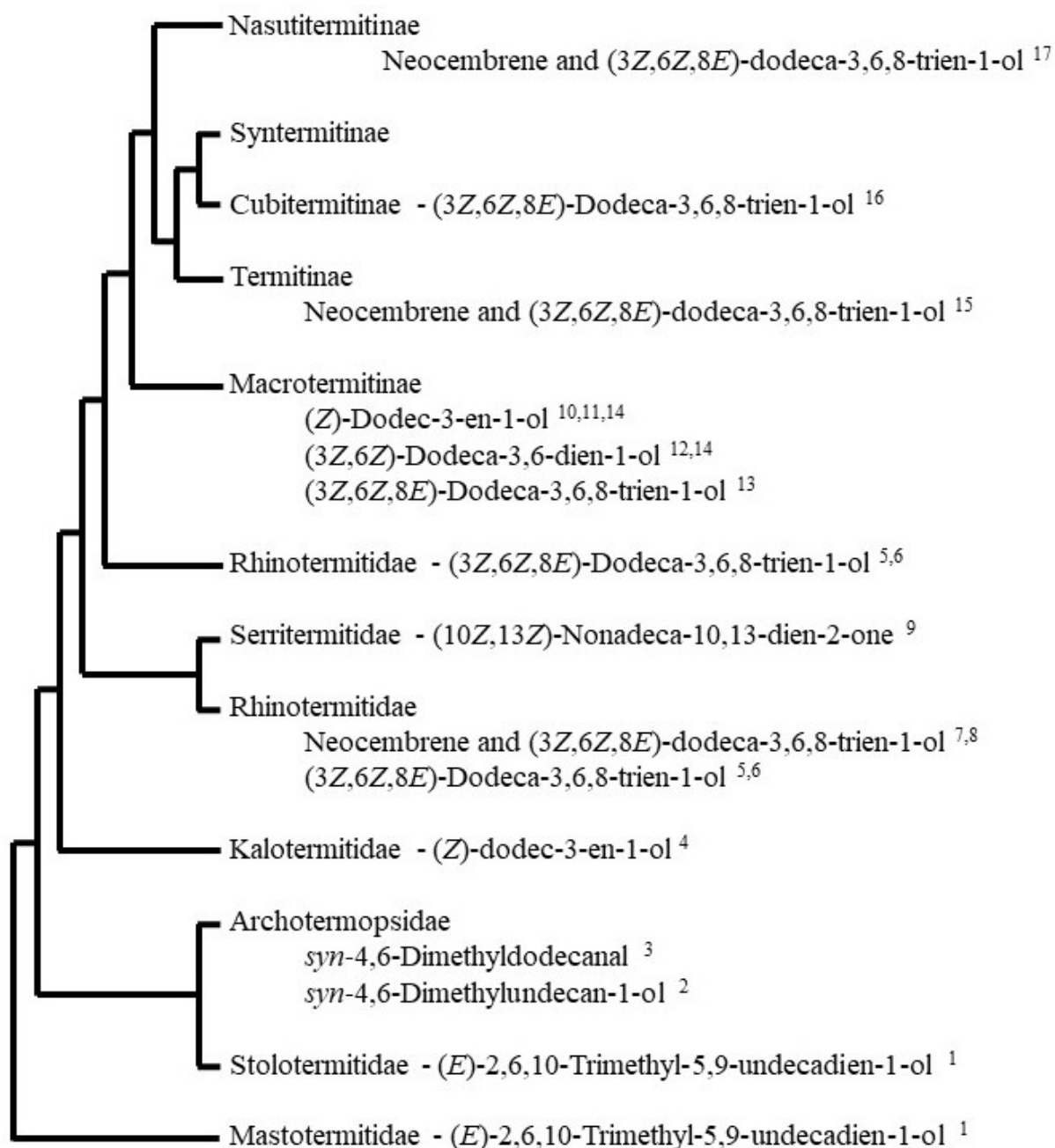
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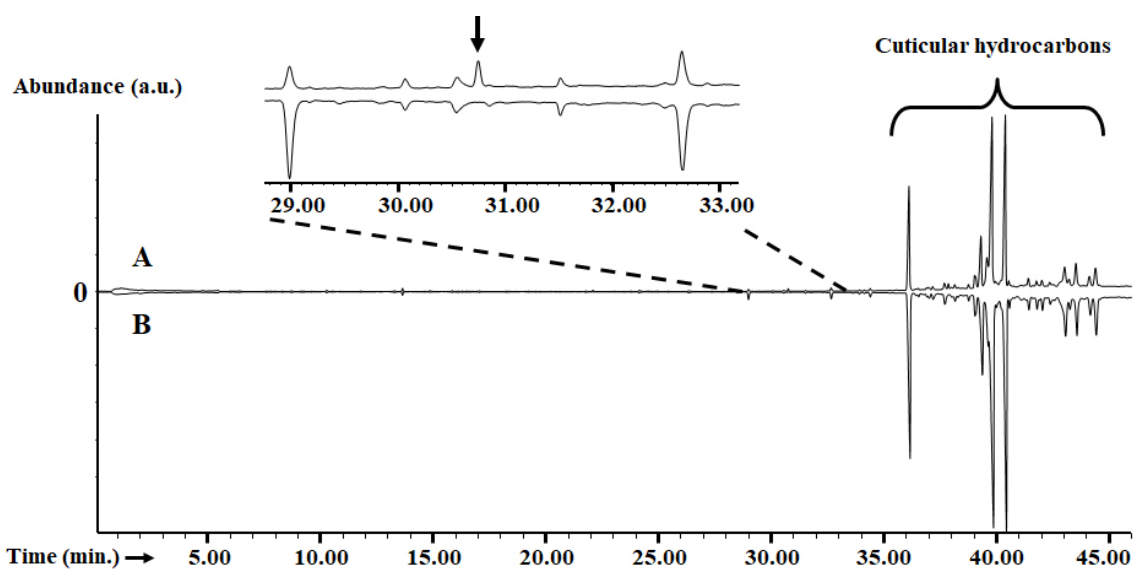
434 **Fig. 1** SIMPLIFIED PHYLOGENY OF THE MAIN TERMITE FAMILIES AND SUB-FAMILIES

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 : ¹Sillam-Dussès et al. 2007; ²Lacey et al. 2011;438 ³Bordereau et al. 2010; ⁴Sillam-Dussès et al. 2009a; ⁵Sillam-Dussès et al. 2006; ⁶Wobst et al.439 1999; ⁷Sillam-Dussès et al. 2005; ⁸Sillam-Dussès et al. 2009b; ⁹Hanus et al. 2012; ¹⁰Peppuy et440 al. 2001a; ¹¹Peppuy et al. 2001b; ¹²Robert et al. 2004; ¹³Bordereau et al. 1993; ¹⁴Wen et al.441 2014; ¹⁵Anani Kotoklo et al. 2010; ¹⁶Sillam-Dussès et al. 2006; ¹⁷Sillam-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-1OL, (3Z,6Z)-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
	(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>Cyrtillitermes 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	χ^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 (χ^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	χ^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 (χ^2 test, NS = non
 473 significant)

474