

## Cuticular hydrocarbons from ants (Hymenoptera: Formicidae) *Odontomachus bauri* (Emery) from the tropical forest of Maranguape, Ceará, Brazil

Hidrocarbonetos cuticulares de formigas (Hymenoptera: Formicidae) *Odontomachus bauri* (Emery) da floresta tropical de Maranguape, Ceará, Brasil

Hidrocarburos cuticulares de hormigas (Hymenoptera: Formicidae) *Odontomachus bauri* (Emery) del bosque tropical de Maranguape, Ceará, Brasil

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### Abstract

Semiochemicals, 5-methyl-nonacosane, Alkanes, Chromatography. Ants are eusocial organisms with great relative abundance and species richness. Studies on these organisms are scarce, especially in the high altitude humid forest environments of the state of Ceará. In view of this condition, an evaluation of the chemical composition of cuticular hydrocarbons (CHCs) of the species *Odontomachus bauri* found and recorded for the first time in this study in the tropical forest of Maranguape was carried out, based on the hypothesis that different nests have different compositions of CHCs. For this purpose, collection incursions were carried out in the region to obtain individuals from three different nests. In the laboratory, the extraction of CHCs was performed by immersing the ants in hexane for 30 minutes. After removing the ants, the extracts were subjected to drying to assess the yield of the samples. Then, the extracts were subjected to a silica gel column, before performing an analysis by gas chromatography coupled with mass spectrometry. The chromatograms obtained were analyzed and demonstrated a great similarity, showing long-chain saturated hydrocarbons, mainly represented by alkanes and methyl-alkanes. 5-Methyl-nonacosane was the component with the highest relative abundance. Statistical analysis of similarity and correlation between samples was performed using non-parametric tests. These analyzes provided sufficient statistical evidence to confirm the existence of a strong positive correlation between the samples and also a significant difference in the composition of HCCs in two of the three paired analyzes performed, thus confirming the study hypothesis.

**Keywords:** Semiochemicals; 5-methyl-nonacosane; Alkanes; Chromatography.

### Resumo

As formigas são organismos eussociais com grande abundância relativa e riqueza de espécies. Estudos sobre esses organismos são escassos, em especial nos ambientes de florestas úmidas de altitude do estado do Ceará. Tendo em vista essa condição, foi realizada uma avaliação da composição química dos hidrocarbonetos cuticulares (CHCs) da espécie *Odontomachus bauri* encontradas e, registradas pela primeira vez neste estudo, na floresta tropical de Maranguape, partindo da hipótese de que ninhos diferentes possuem diferentes composições de CHCs. Para tal, foram realizadas incursões de coleta na região para obter indivíduos de três diferentes ninhos. Em laboratório, foi realizada a extração dos CHCs por imersão das formigas em hexano durante 30 minutos. Após a remoção das formigas, os extratos foram submetidos à secagem para se avaliar o rendimento das amostras. Em seguida, os extratos foram submetidos à coluna de sílica gel, antes da realização de uma análise por cromatografia gasosa acoplada à espectrometria de massas. Os cromatogramas obtidos foram analisados e demonstraram uma grande similaridade, apresentando hidrocarbonetos saturados de cadeia longa, representados principalmente alcanos e metil-alcanos. O 5-

metil-nonacosano foi o componente com maior abundância relativa. A análise estatística de similaridade e de correlação entre as amostras foi realizada por meio de testes não paramétricos. Essas análises forneceram evidência estatística suficiente para confirmar a existência de uma forte correlação positiva entre as amostras e, também, de uma diferença significativa na composição de CHCs em duas das três análises pareadas realizadas, confirmando assim, a hipótese do estudo.

**Palavras-chave:** Semioquímicos; 5-metil-nonacosano; Alcanos; Cromatografia.

### Resumen

Las hormigas son organismos eusociales con gran abundancia relativa y riqueza de especies. Los estudios sobre estos organismos son escasos, especialmente en los ambientes de bosque húmedo de altura del estado de Ceará. Ante esta condición, se realizó una evaluación de la composición química de los hidrocarburos cuticulares (CHCs) de la especie *Odontomachus bauri* encontrada y registrada por primera vez en este estudio en el bosque tropical de Maranguape, con base en la hipótesis de que diferentes nidos tienen diferentes composiciones de CHC. Para ello, se realizaron incursiones de recolección en la región para obtener individuos de tres nidos diferentes. En el laboratorio, la extracción de CHC se realizó sumergiendo las hormigas en hexano durante 30 minutos. Después de retirar las hormigas, los extractos se sometieron a secado para evaluar el rendimiento de las muestras. Luego, los extractos se sometieron a una columna de gel de sílice, antes de realizar un análisis por cromatografía de gases acoplada a espectrometría de masas. Los cromatogramas obtenidos fueron analizados y demostraron una gran similitud, mostrando hidrocarburos saturados de cadena larga, principalmente representados por alcanos y metil-alcanos. El 5-metil-nonacosano fue el componente con mayor abundancia relativa. El análisis estadístico de similitud y correlación entre muestras se realizó mediante pruebas no paramétricas. Estos análisis proporcionaron evidencia estadística suficiente para confirmar la existencia de una fuerte correlación positiva entre las muestras y también una diferencia significativa en la composición de los CHC en dos de los tres análisis pareados realizados, confirmando así la hipótesis del estudio.

**Palabras clave:** Semioquímicos; 5-metil-nonacosano; Alcanos; Cromatografía.

## 1. Introduction

Ants are organisms that play important interactions in ecosystems, acting as fundamental biological indicators in the monitoring of degraded areas thanks to their great diversity, numerical representation and biomass in ecosystems (Lutinski et al., 2017), contributing, for example, to greater quality and reduction of the number of pests in landscapes dedicated to monoculture (Helms et al., 2020; Fernandes et al., 2021).

Ants of the genus *Odontomachus* are predators that build their nests in different strata of the environment such as soil (Cerquera & Tschinkel, 2010), as roots of epiphyte plants (Oliveira et al., 2011) and in rotten branches and trunks deposited in the soil (Brown, 1976). *Odontomachus* ants are commonly found in the oriental (69.2%) and neotropical (30.6%) regions of the world (Ward, 2000), and are known for the rapid closing of their jaws (Gronenberg et al., 1993) and for having a sting pain associated with several cases of urticaria and anaphylaxis (Rodríguez-Acosta et al., 2010).

In *Odontomachus* ants, as well as in other social insects, maintenance and survival of the nests depends on the communication between their individuals. This communication is accomplished through chemical compounds that can trigger various types of behaviors such as recruitment, alarm, defense and nestmate recognition (Wilson, 1965; Holldobler & Wilson, 2009). Recognition between individuals depends on chemical compounds present in the exoskeleton of these animals, the cuticular hydrocarbons (CHCs) and, in some cases, depend on the proportion of volatile compounds present in specific body regions, such as the head and abdomen (Jaffe & Marcuse, 1983).

The cuticle (skin) of all insects is covered with a very thin epicuticular layer of wax (lipid). This cuticle protects the insects from various conditions (e.g., pathogens), but one of the most important features is the protection against transcuticular water loss. In the majority of the insects, the main compounds in the wax layer are hydrocarbons (Drijfhout et al., 2009). These compounds are mainly formed by straight or branched chain alkanes that have a regulating function of cuticular permeability, protecting the animal against dehydration and giving greater adaptability to ambient temperature variations (Blomquist & Bagnères, 2010). CHCs also contribute to the determination of caste and fertility status of individuals (Dietemann et al., 2003; Abdalla et al., 2003), presenting chemical profiles that vary between different species and even between nests of the same

species. This intraspecific diversification is indirectly related to the type of feeding available for the nest (Richard et al., 2004).

CHCs have been characterized in several studies on ants. An analysis of 78 ant species identified almost 1000 CHCs, most of them classified as n-alkanes (97%), monomethyl alkanes (96%), trimethyl alkanes (84%) and alkenes (73%). Monomethyl alkanes are related to communication signals and may be linked to the formation of dimethyl alkanes, specific chemicals that may be characteristic of a species or nest (Martin & Drijfhout, 2009). These characteristics show us the importance of chemical compounds for communication in social insects.

In this work, the species *Odontomachus bauri* (Emery), collected in the tropical forest of Maranguape, was investigated in relation to the composition of CHCs. The tropical forest of Maranguape is located approximately 25 km away from Fortaleza (capital of Ceará) with 920 m high, presenting an area of significant landscape importance, where there are crystalline rocks, including granites, granodiorites, gneisses and migmatites (Tavares et al, 2018). The type of vegetation is characterized as a remnant of tropical forest, as it is formed by a humid and evergreen forest located in the semiarid region of northeastern Brazil. These rainforest enclaves have a positive correlation between the abundance of predators and prey in the region with changes in dominant arthropod species between the rainy and dry seasons. There is also an influence on the abundance and richness of species in response to late rains (Azevedo et al., 2020).

The tropical forest of Maranguape is a rocky grassland mountain which is inserted in a matrix of sandy and stony grasslands, including several vegetation types, such as Cerrado (Brazilian savanna), Atlantic Forest, and Caatinga. The Myrmicinae and Formicinae are the most representative subfamilies of ants in rocky grassland mountains, with 53% and 18% of the total species richness, respectively, but few data were reported about Ponerinae sub-family, which *O. bauri* is included (Costa et al., 2015). Some representatives of Myrmicinae from rocky grassland mountains are the leaf cutters ants *Atta sexdens* Linnaeus (Giesel et al., 2020; 2021) and as example of ants with sub-family Formicinae, Nakano et al. (2013) reported the occurrence of *Myrmelachista* Roger in the Atlantic Forest of Southeastern Brazil and *Lachnomyrmeis nordestinus* was found in tropical forest of Maranguape (Feitosa & Brandão, 2008). The characterization of the types of ants that inhabit this mountain is scarce. Ants of the genus *Odontomachus* have already been identified in humid highland forests of Ceará, having been seen in the tropical forest of Baturité (Azevedo Filho et al., 2003; Hites et al., 2003). This will be the first report of the presence of ants of the genus *Odontomachus* in the tropical forest of Maranguape. Therefore, this work aimed to evaluate the hypothesis that ants from different nests have different compositions of CHCs and, for that, *O. bauri* ants were collected in the northeastern region of Brazil to obtain the characterization of their CHCs.

## 2. Methodology

### Insect materials

A qualitative-quantitative case study (Pereira et al., 2018) was carried out and, for that, *O. bauri* ants were collected in an area of the tropical forest of Maranguape (CE, Brazil) (3°52'03.4"S 38°41'44.1"W; MMA/ICMBIO/SISBIO authorization: 58972-1). Ants were collected during the 2016 rainy season, in March and April. The rainfall and thermal average of the period was 176 mm and 23 °C, respectively. The nests were located at an altitude of 300 m and at least 300 m apart. Ants from three different nests were collected by aspiration and stored in separate containers which were then taken directly to the laboratory for CHCs extraction. Two hundred individuals from each container were selected for the extraction of cuticular hydrocarbons, thus composing three distinct groups. Only workers were used in the study, providing a sufficient number of individuals of the same caste to remove the extract. The extraction of CHCs by immersion in solvent was performed based on the studies carried out by Menzel et al. (2017), with modifications. Each group was separately immersed in hexane for 30 minutes at room temperature (25°C). After this time, the ants were removed by filtration, leaving behind a hexane extract. The extract was subjected to rotary evaporation for complete solvent removal, forming a dry mass for analysis by gas chromatography coupled

to mass spectrometry (GC-MS).

### Analysis of Extracts

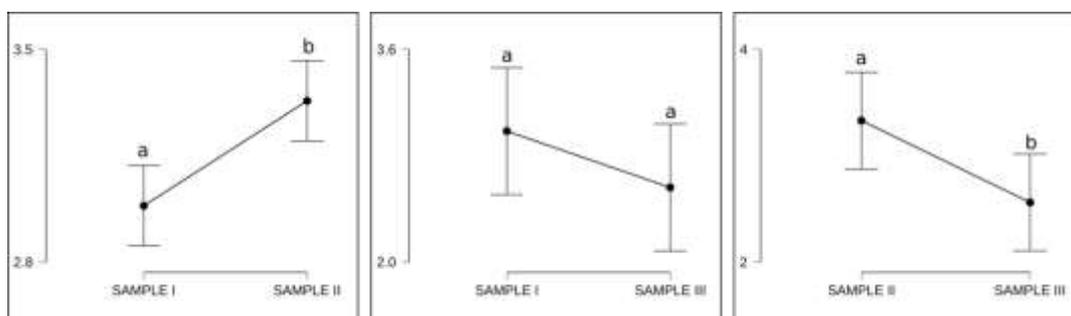
The obtained extracts from ant samples were passed through a silica gel (60 mesh) chromatography column, being eluted with hexane, to exclude polar minor compounds before being sent for CG-MS. The analysis was performed in a Shimadzu QP-5050 instrument with a DB-1 fused silica capillary column with dimethylpolysiloxane (30m x 0.25mm ID x 0.25 $\mu$ m film); Injection initial temperature: 25° C; Interface temperature: 230° C; Control Mode: Split; Split Ratio: 1:27; Column inlet pressure: 100 kPa; Column flow: 1.7 mL/min; Total Flow: 50.0 mL/min; Equilibrium Time: 1.00 min; Linear Velocity: 47.4 cm/sec; Temperature was 35 to 180° C at 4° C /min, then 180 to 280° C at 17° C /min and 280° C for 10 min; The mass spectrum was obtained by electron impact at 70 eV. Hydrocarbons were identified using a library search (NIST08), the diagnostic fragmented ions and the Kovats indices.

## 3. Results

### Statistical Analysis

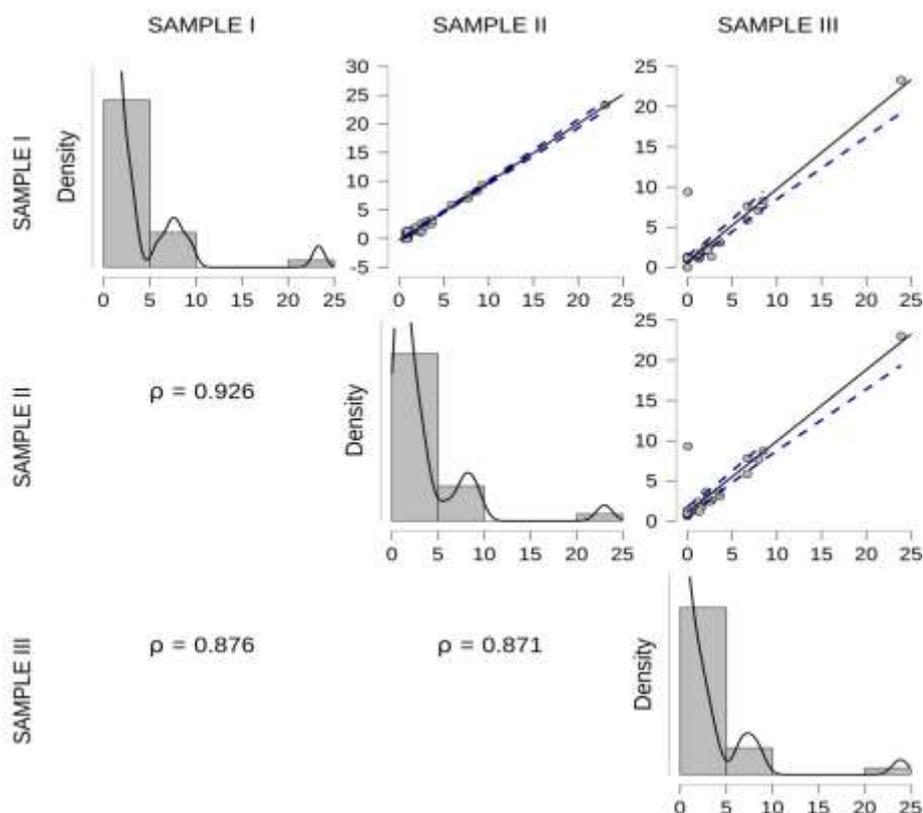
The relative abundance data of the CHCs were organized in a matrix, thus allowing a comparative statistical analysis. The analyzes were performed using the JASP Team software (2021, version 0.14.3). The normality of the data was assessed by the Shapiro-Wilk test ( $\alpha = 0.05$ ). The Shapiro-Wilk test of three samples showed significant values ( $p < 0.001$ ) and, for this reason, the null hypothesis ( $H_0$ ) was rejected. Therefore, the relative abundance data of the samples do not have a normal distribution. In view of this condition, statistical analysis was performed based on non-parametric tests. To this end, the Wilcoxon signed-rank (paired) and Spearman (correlation) tests were conducted. The three samples were considered as replicates. The Wilcoxon signed-rank test proved to be significant when comparing samples I-II ( $p = 0.003$ ) and II-III ( $p < 0.001$ ), thus rejecting the  $H_0$ . Therefore, there is sufficient statistical evidence to affirm that there are differences in the comparisons between samples I-II and II-III (Figure 1). The comparison between samples I-III was not significant ( $p = 0.254$ ), that is, there was no rejection of  $H_0$ . Therefore, there is sufficient statistical evidence to state that samples I and III are the same. The Spearman's test (Figure 2) showed a strong positive correlation between the samples (Samples I-II: 0.926; Samples II-III: 0.871; Samples I-III: 0.876,  $p < 0.001$ ).

**Figure 1.** Descriptive plots of Wilcoxon signed-rank test between the samples I, II and III. (a; b) Indicators of statistically significant difference ( $\alpha = 0.05$ ).



Source: Authors.

**Figure 2.** Correlation plots of Spearman's test between the samples I, II and III ( $\alpha = 0,05$ ). (p) Sperman's non-parametric coefficient demonstrating the existence of a strong positive correlation between the samples.



Source: Authors

### Extract Yield and Analysis of Gas Chromatography Coupled to Mass Spectrometry

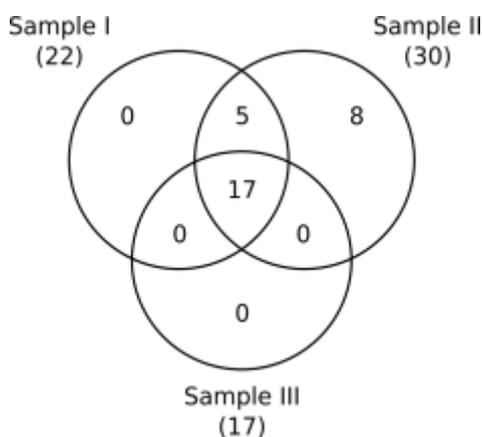
The extraction method presented a low yield, less than 1% (Table 1). Each sample of ants was composed of 200 workers. The gas chromatograms of the three samples showed similar and very consistent patterns. Samples I and II showed 30 peaks, while sample III showed 27 peaks (Figure 4). The identification of components was tentatively deduced by comparing the mass spectra (Adams, 2012) and the Kovat's Index obtained by the linear regression analysis ( $y = 40,082x + 560,59$ ;  $R^2 = 0,84622$ ), using the main normal chain hydrocarbon constituents. A large amount of long chain hydrocarbons (C15-C36) was observed in the samples (Table 2).

**Table 1.** Dry weight of *Odontomachus bauri* ants of Maranguape (CE, Brazil) and their extracts.

Samples	Ants (n= 200)	Extract (mg)	Yield (%)
I	2.2423 g	13,7	0.614
II	3.0379 g	20,5	0.674
III	2.5798 g	23,5	0.910

Source: Authors.

**Figure 3.** Venn diagram showing composition similarity between samples I, II and III obtained from *Odontomachus bauri* ants of Maranguape (CE, Brazil).

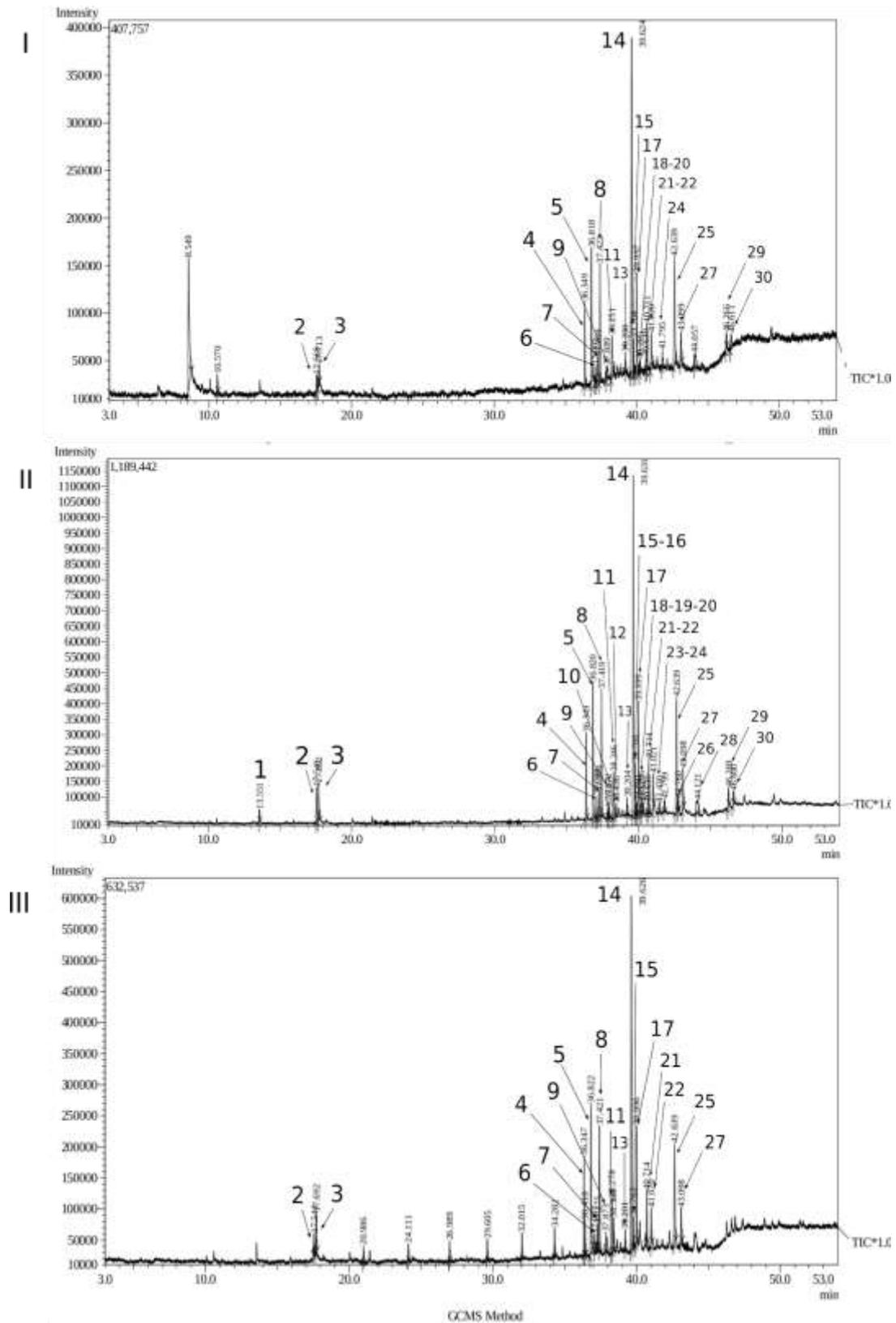


Source: Authors.

The compounds 1-hexadecyne, (Z)-8-heptadecene, heneicosene, docosane, 2-methyltetracosane, pentacosane, hexacosane, heptacosane, 7-methylheptacosane, nonacosane, 5-methylnonacosane, 3-methylnonacosane, triacontane, hentriacontane, dotriacontane, tritriacontane and tetratriacontane were similar to the three samples (56.66%), whereas the compounds 12-methyltriacontane, 11,20-dimethyltriacontane, 8,20-dimethyldotriacontane, 7-methylpentatriacontane, and hexatriacontane were observed in samples I and II (16.66%).

Eight (26,66%) components (pentadecane, 11-methylheptacosane, octacosane, 2-methylnonacosane, 10-methyltriacontane, 6-methyldotriacontane, 3-methyltritriacontane and 8-methyltetratriacontane) were observed only in sample II (Figure 3), mostly methyl alkanes. The 5-methylnonacosane component showed the highest mean relative abundance of the samples ( $23.41\% \pm 0.43$ ), followed by triacontane ( $7.55 \pm 0.48$ ), tritriacontane ( $7.37\% \pm 0.60$ ), docosane, ( $6.25\% \pm 5.36$ ) and heneicosane ( $6.13\% \pm 0.48$ ).

**Figure 4.** Chromatograms of samples I, II and III obtained from *Odontomachus bauri* ants of Maranguape (CE, Brazil).



Source: Authors.

**Table 2.** List of cuticular hydrocarbons found in *Odontomachus bauri* ants of Maranguape (CE, Brazil). Compounds listed by Kovats index (KI) order, showing the average (AVG) of relative abundance (RA) and standard deviation (SD) between samples. (X) Presence indicator. (\*) Component with major relative abundance.

Hydrocarbons	Formula	Class	KI	RA	Samples			
				AVG% ± SD	I	II	III	
1.	Pentadecane	C <sub>15</sub> H <sub>32</sub>	Alkane	1500	0.28 ± 0.48		x	
2.	1-Hexadecyne	C <sub>16</sub> H <sub>30</sub>	Alkyne	1664	1.57 ± 0.73	x	x	x
3.	8-Heptadecene	C <sub>17</sub> H <sub>34</sub>	Alkene	1676	2.18 ± 0.75	x	x	x
4.	Heneicosane	C <sub>21</sub> H <sub>44</sub>	Alkane	2100	6.13 ± 0.48	x	x	x
5.	Docosane	C <sub>22</sub> H <sub>46</sub>	Alkane	2200	6.25 ± 5.36	x	x	x
6.	2-Methyltetracosane	C <sub>25</sub> H <sub>52</sub>	Methyl Alkane	2465	1.31 ± 0.23	x	x	x
7.	Pentacosane	C <sub>25</sub> H <sub>52</sub>	Alkane	2500	1.71 ± 0.14	x	x	x
8.	Hexacosane	C <sub>26</sub> H <sub>54</sub>	Alkane	2600	8.48 ± 0.23	x	x	x
9.	Heptacosane	C <sub>27</sub> H <sub>56</sub>	Alkane	2700	1.18 ± 0.12	x	x	x
10.	11-Methylheptacosane	C <sub>28</sub> H <sub>58</sub>	Methyl Alkane	2733	0.25 ± 0.43		x	
11.	7-Methylheptacosane	C <sub>28</sub> H <sub>58</sub>	Methyl Alkane	2746	3.26 ± 0.32	x	x	x
12.	Octacosane	C <sub>28</sub> H <sub>58</sub>	Alkane	2800	0.32 ± 0.55		x	
13.	Nonacosane	C <sub>29</sub> H <sub>58</sub>	Alkane	2900	1.29 ± 0.10	x	x	x
14.	5-Methylnonacosane*	C <sub>30</sub> H <sub>62</sub>	Methyl Alkane	2950	23.41 ± 0.43	x	x	x
15.	3-Methylnonacosane	C <sub>30</sub> H <sub>62</sub>	Methyl Alkane	2958	2.74 ± 0.79	x	x	x
16.	2-Methylnonacosane	C <sub>30</sub> H <sub>62</sub>	Methyl Alkane	2972	0.31 ± 0.53		x	
17.	Triacotane	C <sub>30</sub> H <sub>62</sub>	Alkane	3000	7.55 ± 0.48	x	x	x
18.	12-Methyltriacotane	C <sub>31</sub> H <sub>64</sub>	Methyl Alkane	3034	0.67 ± 0.58	x	x	
19.	10-Methyltriacotane	C <sub>31</sub> H <sub>64</sub>	Methyl Alkane	3035	0.22 ± 0.38		x	
20.	11,20-Dimethyltriacotane	C <sub>32</sub> H <sub>66</sub>	Dimethyl Alkane	3068	0.22 ± 0.38	x	x	
21.	Hentriacotane	C <sub>31</sub> H <sub>64</sub>	Alkane	3100	3.47 ± 0.21	x	x	x
22.	Dotriacotane	C <sub>32</sub> H <sub>66</sub>	Alkane	3200	2.48 ± 0.09	x	x	x
23.	6-Methyldotriacotane	C <sub>33</sub> H <sub>68</sub>	Methyl Alkane	3245	0.21 ± 0.37		x	
24.	8,20-Dimethyldotriacotane	C <sub>34</sub> H <sub>70</sub>	Dimethyl Alkane	3265	0.70 ± 0.66	x	x	
25.	Tritriacotane	C <sub>33</sub> H <sub>68</sub>	Alkane	3300	7.37 ± 0.60	x	x	x
26.	3-Methyltritriacotane	C <sub>34</sub> H <sub>70</sub>	Methyl Alkane	3376	0.28 ± 0.48		x	
27.	Tetratriacotane	C <sub>34</sub> H <sub>70</sub>	Alkane	3400	2.40 ± 0.34	x	x	x
28.	8-Methyltetratriacotane	C <sub>35</sub> H <sub>72</sub>	Methyl Alkane	3440	0.64 ± 0.56		x	
29.	7-Methylpentatriacotane	C <sub>36</sub> H <sub>74</sub>	Methyl Alkane	3540	0.82 ± 0.71	x	x	
30.	Hexatriacotane	C <sub>36</sub> H <sub>74</sub>	Alkane	3600	0.71 ± 0.63	x	x	
				Alkanes	49.32			
				Methyl Alkanes	34.12			
				Dimethyl Alkanes	0.92			
				Alkenes	2.18			
				Alkyne	1.57			
				Unidentified	11.89			

Source: Authors.

#### 4. Discussion

The comparative analysis of the obtained compounds of *O. bauri* ants revealed the existence of a great variety of CHCs comparing the samples. Molecules with 15 to 36 carbons have been identified. In absolute numbers, 14 (46.66%) compounds are n-alkanes and 12 (40%) are monomethyl alkanes. Based on the sum of the averages of the relative abundances, 49.32% are alkanes, 34.12% are methyl alkanes, 0.92% are dimethyl alkanes, 2.18% are alkenes and 1.57% are alkynes. These results corroborate the patterns of hydrocarbon predominance observed in ants (Martin & Drijfhout, 2009).

The high proportion of n-alkanes can be interpreted as consistent according to the species' nesting pattern, known for building nests under branches and trunks deposited in the soil (Brown, 1976), which favors greater susceptibility of individuals to climatic conditions. The northeastern region of Brazil is known for its semi-arid condition, with dry characteristics and high temperatures (Marengo et al., 2018). High temperature and low humidity contribute to a differentiation of the cuticular layer, promoting a greater relative abundance of n-alkanes in its composition. (Wagner et al., 2001).

Six of the 12 methyl alkanes found are exclusive of sample II (Table 2), demonstrating the existence of an intraspecific variation in the population, which is in accordance with the statistical results obtained. This distinction is possibly related to the geographic distance of the nests and the availability of resources for them. The intraspecific differences of CHCs have already been observed in several insect species and may be related to feeding variations and nest substrate type (Richard & Hunt, 2013). The diet of ants has the ability to change the composition of CHCs, which affects the recognition ability between individuals (Liang & Silverman, 2000).

The recognition ability is related to the aggressive behavior presented against individuals that do not belong to the nest. This aggressiveness can be reduced when neighboring nests are submitted to the same diet (Buczowski, 2004). The similarities between samples I and III may be a consequence of geographical proximity or the same availability of food in the environment in which the nests are found, thus contributing to the recognition and reduction of aggressiveness among individuals. Studies carried out with the species *Pachycondyla analis* (Latreille) have shown that n-alkanes remain consistent in the cuticle profiles of different nests, while methyl alkanes and alkenes are possibly related to the ability to recognize among individuals (Yusuf et al., 2010).

In this study, the 8-heptadecene component was observed in the three samples, indicating that the extraction methodology obtained organic compounds from other regions of the animal's body besides the cuticle. This component has been previously identified in the Dufour gland of ants *O. bauri* (Morgan et al., 2003) and also in ants of the genus *Diacamma* as a recruitment pheromone (Fujiwara-Tsujii et al., 2012). Nevertheless, the *Odontomachus* ants do not form trails and the search for food occurs in a solitary way with the orientation based on the canopy of the trees (Oliveira & Hölldobler, 1989), then the component 8-heptadecene is possibly related to recruitment capacity among the nest individuals.

Taken into account a wide spectrum of studies, it appears clearly that the main role of the n-alkanes is to control transcuticular water movement while the unsaturated compounds and methyl-branched hydrocarbons are more likely to be involved in communication (Drijfhout et al., 2009). CHCs profiles are well known in the literature as a method of differentiating varieties into insects and may vary quantitatively and qualitatively in species (Blomquist & Bagnères, 2010). In ants of the genus *Odontomachus* is similar. The major component of the cuticle of these animals acts as an indicator of fertility. For example, in *O. brunneus* (Patton) ants, a component that acts as a fertility signal in the species, the (Z)-9-nonacosene was identified. This component is used to differentiate reproductive and non-reproductive females in the nest. (Smith et al., 2012). A pattern that has been conserved in the species despite the existence of intraspecific variations of cuticular profiles of the population (Smith et al., 2013). Males of the species have another signaling component, (Z)-9-pentacosene (Smith et al., 2014).

In species *O. ruginodis* (Smith), queens differ from workers due to the presence of a specific cuticular component, 2,5-dialkyltetrahydrofurans. In *O. relictus* (Deyrup & Cover) and *O. haematodus* (Linnaeus) species, queens are distinguished

from workers by increasing the relative abundance of methyl alkanes and dimethyl alkanes. *O. relictus* has great abundance of the (3,9-; 3,7-) dimethylheptacosane component, while in *O. haematodus* the same is true for the (13-; 11-; 5-) methylheptacosane, and 5,19-dimethylheptacosane. In all three species, males have distinct cuticular profiles from females, characterized by increased relative abundance of alkanes, alkenes and dienes (Smith et al., 2016).

In *O. hastatus* (Fabricius), distinct cuticle profiles were observed between queens, workers and foundresses (unrelated ants). The workers have a large proportion of methyl alkanes (33.7%) and dimethyl alkanes (25.92%), while queens have a higher relative proportion of alkadienes (37.94%) and methyl alkenes (24.61%). The foundresses presented higher relative abundance of methyl alkanes (28.8%) and methyl alkenes (22.19%). The most abundant component in workers and queens was (15-; 17-) methylnonatriacontane, while in the foundresses it was the 5-methylheptatriacontane component (Berthelot et al., 2017). Methyl-branched alkanes were active in several physiological aspects, functioning as sex pheromones, kairomones (a signal molecule that benefits the receiver but disadvantages the donor) and anti-aphrodisiacs (Drijfhout et al., 2009).

In *O. bauri* samples the 5-methylnonacosane component showed a high relative abundance and probably play a role in the communication as a sex attractant of the species, contributing to the nest caste characteristic. In addition, two other molecules with chains of the same length and different branch points were observed, 3-methyl- and 2-methylnonacosane. This variation in the methylation points may be related to the intensification of the ants' signaling capacity. A study with the ant *Linepithema humile* (Mayr) showed that the species is able to distinguish different branching points in molecules with chains with same length more easily than in molecules with chains of different sizes and equal branching points (van Wilgenburg, 2012).

The 5-methylnonacosane component was found in less relative abundance in the cuticular profile of the species *O. relictus* (Smith et al., 2016; Smith, 2019). However, the component indicated as a marker of fertility of the species was (Z)-9-nonacosene (Smith et al., 2016). This same component was identified in the post-pharyngeal gland secretions of *O. bauri* ants collected in Venezuela, also showing a small relative abundance (Sainz-Borgo, 2011). Given this information, this is the first report of this cuticular component with high relative abundance in the species, as well as the record of its cuticular profile in northeastern Brazil.

## 5. Conclusion

The extract samples show great similarity in the composition of the CHCs, a condition reinforced by the Spearman correlation analysis. Wilcoxon's paired analysis between the extracts confirmed the hypothesis that the nests have different compositions in two of the three pairings. Pairings I-II and II-III proved to be statistically distinct. However, the same did not occur in the pairing between samples I-III, being therefore considered statistically equal. This condition may be a reflection of the proximity between the nests and the availability of food in the region. Future studies can be conducted to assess the effect of distance on the composition of CHCs between nests. The 5-methyl-nonacosane component had the highest relative abundance in all samples, being this the first representative record of this component in the species. Based on information obtained from several studies on ant CHCs, it is observed that the major components found in the cuticle of these animals act as chemical signals that identify the fertile and non-fertile individuals in the nest. Thus, the 5-methyl-nonacosane component is possibly the chemical signal for infertile females in the nest. However, future studies must be carried out to confirm this hypothesis.

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