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Variation in Chemical Composition of Cuticular and Nonpolar Compounds of Venom of *Apoica pallens* and *Polistes versicolor*

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Abstract

Although cuticular hydrocarbons and venom are important to the evolutionary success of social behavior, studies that investigated these compounds in tropical social wasps are rare. Thus, the aim of this study was to compare the cuticular chemical composition and the nonpolar portion of venom of *Apoica pallens*, a swarm-founding wasp and *Polistes versicolor* an independent-founding wasp. Gas chromatography coupled to mass spectrometry (GC/MS) technique was used. In the samples of *A. pallens*, 66 compounds were identified on the cuticle and 87 in venom, 13 are unique of the cuticle and 26 of venom. In the samples of *P. versicolor*, 85 compounds were identified on the cuticle and 60 in venom, 10 are exclusive of the cuticle and 5 of venom. The results show that, although they present different foundation types and organize in colonies with significantly different population number, the variation in chain length of compounds is relatively similar. In addition, in both types of samples of both species, the most representative class of compounds in content and number are the branched alkanes, which are recognized as the most effective during interactions between nestmates. However, there is greater similarity in content of shared compounds between samples of cuticle and venom of *A. pallens*, suggesting that because it is a species that is organized in more populous colonies, it may have a more elaborate signaling system based on volatile compounds of venom.

Introduction

The Hymenoptera is one of the largest orders of insects, which comprises social wasps, bees and ants, among others. In insects' societies, it was necessary to evolve a mechanism to keep the cohesion between their members, mostly in the form of chemical compounds used as signals exchanged during interactions, called semiochemicals. Among these compounds, the cuticular hydrocarbons (CHCs), which are constituents of the lipid layer of insects' cuticle, stand out (Lockey, 1988).

The CHCs act as contact or surface pheromones, as already reported in some studies (Abdalla et al., 2003; Neves et al., 2012; Bello et al., 2015), and as signals or clues for nestmates, allowing the identification of conspecifics, assisting in maintenance of colonial structure, distinguishing individuals according to their function, physiological status and hierarchical rank (Provost et al., 2008), acting as a specific chemical signature of the individual.

These surface pheromones are mainly composed of hydrocarbons, especially linear alkanes, branched alkanes and alkenes (Devigne & Biseau, 2012). The primary functions of

CHCs are to prevent water loss and act as a protective coating for insects (Gibbs, 1998). In addition, they mediate intra and interspecific interactions between these insects (Blomquist & Bagneres, 2010). It is known that CHCs vary significantly between species (Antonialli-Junior et al., 2008; Ferreira et al., 2012; Santos & Nascimento, 2015), castes (Nunes et al., 2009, Ferreira-Caliman et al., 2013) and nestmates (Lorenzi et al., 2004; Costanzi et al., 2013), besides signaling dominance and fertility (Cuvillier-Hot et al., 2001; Van Oystaeyen et al., 2014), and may also vary according to the insect's age (Abdalla et al., 2003; Biseau et al., 2004; Antonialli-Junior et al., 2007; Nunes et al., 2009).

Social insects are also able to synthesize compounds for venom production, which represents part of a mechanism to capture prey and defend their colonies, and can also act on communication, as described by Mateus (2011), which assessed that the wasp *Parachartergus fraternus* uses venom to mark a new site for colony foundation before the start of migration. Therefore, it can be inferred that there are elements in venom composition that must act as signals exchanged during interactions between conspecifics, probably those lighter and volatile among all compounds found in venom. Bruschini et al. (2006a) found pheromones in the volatile portion of *P. dominula* venom that play a communicative role by inducing alarm behavior. Behavioral studies with venom extracts (Bruschini et al., 2008) also revealed that *P. dominula* wasps are more stimulated by venom of workers than foundresses. In addition, Post and Jeanne (1984) evaluated the potential role of volatile components of venom of *Polistes* female wasps as a sex pheromone, attractive to males. Thus, the study on volatile components of venom is also important to understand better which type of compounds are used for exchange of signals between conspecifics in colonies of social wasps.

Apoica pallens (Fabricius, 1804) is a social swarm-founding wasp of nocturnal habit and thus presents large compound eyes and ocelli that are adaptations to seeing in dark (Schremmer, 1972). This species is found from Mexico to northeastern Argentina (Richards, 1978) and their colonies have from hundreds to thousands of individuals. *Polistes versicolor* (Olivier, 1791) is an independent-founding wasp of diurnal habit, whose nests have a single uncovered comb attached to the substrate by a peduncle. Their colonies are relatively small in number of individuals and are very common in urban areas, being abundant in South America, present from Costa Rica to southern Brazil and Argentina (Richards, 1978).

Despite CHCs and venom being recognized as important compounds to the evolutionary success of social behavior, and that there is a relatively significant number of studies about them, still are rare those that investigated the cuticular composition and nonpolar portion of venom (Sledge et al., 1999; Dani et al., 2000; Bruschini et al., 2006b; Bruschini et al., 2008; Silva et al., 2016) in tropical social wasps species. Thus, the aim of this study was to compare the chemical composition of cuticle and nonpolar portion of venom of *Apoica pallens*, a swarm-founding wasp and *Polistes versicolor* an independent-founding wasp.

Materials and methods

Samples collection and extraction of cuticular compounds and nonpolar compounds of venom

A total of 110 females of *A. pallens* from a single colony were used, all workers with the same approximate age (older), determined by the method of apodeme coloring (Richards, 1971; West-Eberhard, 1973) and 310 females of *P. versicolor* from 3 colonies, all with the same approximate age.

Colonies of both species were collected in rural and forest areas in the surroundings of Dourados-MS, Brazil (22°14′38.8″S, 54°49′36.6″W) in February 2015 and April 2016, respectively. The collections were performed using plastic bags and ether, by involving their nests and subsequently detaching them from the substrate. Then, the wasps were transferred to the laboratory, where they were anesthetized at low temperature for subsequent extraction.

For extraction of CHCs, 10 workers of each species, totaling 10 samples of each species were used. Extraction of CHCs of the whole individuals was performed without any kind of fixative. Each sample was immersed in a glass container with 2 ml of hexane for 2 minutes. After solute removal, samples were dried under fume hood and frozen for a maximum of 30 days. For chromatographic analysis, each extract was solubilized in 200 µl of hexane (Tedia, HPLC grade).

For characterization of the nonpolar portion of *A. pallens* venom, 10 readings were performed, each sample with the content of 10 venom reservoirs. For venom samples of *P. versicolor* a triplicate was performed, each with the content of 100 reservoirs. This number varied between the two species due to their body size, and consequently of their venom reservoir. The definition of number of reservoirs was performed using preliminary tests carried out on samples from each species.

Venom reservoir extraction was performed by dissection, in ultra-pure water in order to prevent that membrane compounds were extracted, with the aid of tweezers and stereomicroscope, and subsequent removal of glandular filaments and sting. To extract the venom, the reservoir was lightly pressed into a vial until the release of its content. During the entire procedure, all samples were kept on ice to prevent volatilization and degradation of lighter compounds. Then, samples were subjected to extraction in hexane followed by ethyl acetate. For each sample, 200 µL of ultra-pure water and 200 µl of hexane (HPLC grade) were added, followed by agitation for 30 seconds and rest for three minutes, later the phases were separated. In the aqueous fraction 500 µL of ethyl acetate (HPLC grade) were added, followed by agitation for 30 seconds and rest for 10 hours. After phase separation, the ethyl acetate fraction was joined to the hexane fraction of each sample, dried under fume hood, and solubilized in 200 µl of hexane.

Analysis of samples by gas chromatography coupled to mass spectrometry (GC/MS)

Samples were analyzed employing a gas chromatograph coupled to a mass spectrometer (GC-MS Ultra 2010, Shimadzu, Kyoto, Japan), using a fused silica capillary column DB-5 (J & W, Folsom, California, USA) with 30 m length x 0.25 mm diameter x 0.25 μm thickness. The analysis conditions, programming of column temperature and scanning parameters were the same described in the study by Paula et al. (2018).

The identification of compounds was performed employing the calculated retention index (Van den Dool & Kratz, 1963), using a mixture of linear alkanes (C_7-C_{40}, Sigma Aldrich with purity ≥ 90%) and the linear alkanes were employed as standard for identification of compounds. These calculated indexes were compared with the literature retention indexes (Brown et al., 1991; Howard et al., 2001; Zhu et al., 2006; Moore et al., 2014; Weiss et al., 2014; Silva et al., 2016), associated with interpretation of mass spectra obtained for the samples and compared with databases (NIST21 & WILEY229). Based on the methodology described by Dapporto et al. (2004, 2005), the area of each peak of the chromatogram of each sample was transformed into percentage. In addition, the compounds representing less than 0.5% were not presented in the tables. The major compounds were considered those that represented at least 4% of the total relative area.

To assess the relationship between cuticle and venom of these two species, a discriminant function analysis (DFA) was employed using only the compounds shared by the 4 groups, in which Wilk's Lambda near 0 reveals that the groups do not overlap, i.e., are different, and values close to 1 show overlap.

Results

In samples of *A. pallens* cuticle 74 peaks were detected, 66 of these were identified (89.2%) with carbonic chain length varying from C_{16} to C_{37}. The five major compounds in these samples were 13-methylheptacosane (15.56%) Heptacosane (13.09%); x-methylheptacosane (10.83%); 13-methylhentriacontane (8.32%); 13-methylpentacosane (7.17%) (Table 1). The most numerous and abundant classes of compounds were branched alkanes representing 74.5% of compounds, linear alkanes 23.5% and alkenes 1.6%.

In samples of chemical profile of venom of this species 97 peaks were detected, of these 87 were identified (89.7%) ranging from C_{15} to C_{37}. The five major compounds were 13-methylheptacosane (9.73%), 13-methylpentacosane (7.65%), 11,15-dimethylpentacosane (5.24%), 6-methyloctacosane (4.67%), x-methylheptacosane (4.95%) (Table 1). Branched alkanes were the most significant regarding number of compounds and abundance, representing 77.5% of compounds, followed by linear alkanes 8%, and alkenes 7.5% (Figs 1B and 2B).

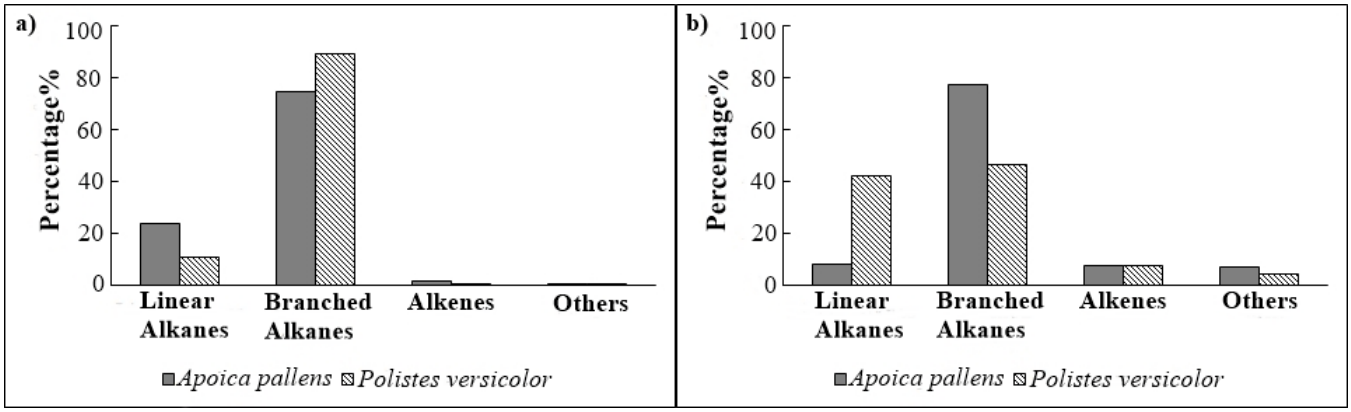

Fig 1. Mean percentage of different classes of compounds in a) cuticular chemical profile and b) venom chemical profile of *Apoica pallens* and *Polistes versicolor*.

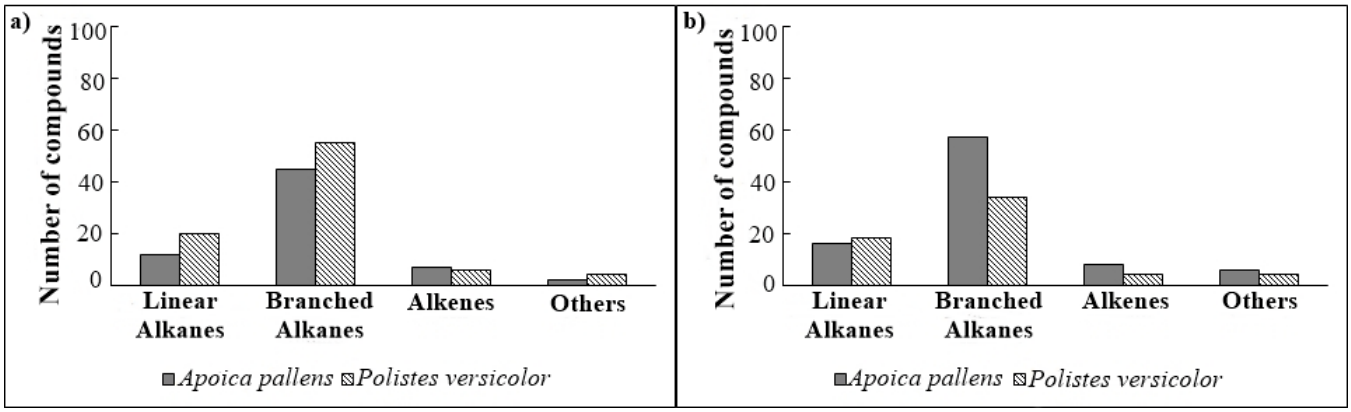

Fig 2. Number of compounds of the different classes in a) cuticular chemical profile and b) venom chemical profile of *Apoica pallens* and *Polistes versicolor.*

Table 1. Percentage area of nonpolar compounds (> 0.5%) present in cuticle and venom of eusocial wasps *Apoica pallens* and *Polistes versicolor*.

Calculated index (kraftz)	Compounds	*Apoica pallens* Cuticle	*Apoica pallens* Venom	*Polistes versicolor* Cuticle	*Polistes versicolor* Venom
		Percentage (%±standard deviation)			
1500	Pentadecane	ND	0.36±0.61	0.04±0.02	4.91±4.53*
1568	(*R*)-(-)-Mellein	ND	0.63±1.48	ND	ND
1600	Hexadecane§	0.02±0.02	0.19±0.60	0.02±0.01	4.74±2.08*
1658	x-Methylhexadecane	ND	3.52±4.31	ND	ND
1662	Heptadecadiene	ND	0.29±0.69	0.04±0.05	2.36±2.18
1699	Heptadecane	ND	0.34±0.60	0.01±0.01	2.49±0.81
1734	4- Hidroximellein	ND	0.58±1.64	ND	ND
1744	x-Methylheptadecane	ND	0.65±1.47	ND	ND
1774	3-Methylheptadecane	ND	0.58±1.45	ND	ND
1793	Octadecene	ND	0.32±0.99	0.03±0.02	ND
1800	Octadecane	ND	0.06±0.09	0.11±0.03	11.75±1.48*
1822	2-Methyl-6-undecenyl piperidine	ND	3.60±3.51	ND	ND
1826	x-Methyloctadecane	ND	1.05±2.70	0.03±0.05	4.11±1.93
1874	Nonadecene	0.07±0.11	0.67±0.70	ND	1.33±0.17
1900	Nonadecane	ND	ND	0.01±0.00	0.72±0.20
1918	x-Methylnonadecane	TR	0.56±0.71	ND	ND
1926	x-Methylnonadecane	TR	0.01±0.04	ND	0.59±0.18
1952	x-Methylnonadecane	ND	2.39±4.50	ND	ND
1967	2-Methylnonadecane	ND	2.91±5.63	ND	3.39±0.44
1978	3-Methylnonadecane	ND	3.58±3.12	ND	ND
1982	Eicosene	ND	0.05±0.08	0.02±0.04	4.33±1.91*
2001	Eicosane	ND	0.16±0.29	0.02±0.00	1.00±0.30
2116	x-Methylheneicosane	ND	ND	0.01±0.01	0.56±0.23
2131	7-;9-;11-Methylheneicosane	ND	2.24±2.60	0.02±0.03	0.64±1.11
2172	x-Methylheneicosane	ND	ND	0.58±1.00	1.34±0.29
2273	Tricosadiene§	0.02±0.06	0.63±1.99	0.09±0.17	1.04±0.69
2278	Tricosene§	0.01±0.04	2.46±2.50	0.02±0.03	0.44±0.76
2300	Tricosane§	0.08±0.10	0.19±0.27	0.10±0.16	1.95±0.43
2394	x-Tetracosene	ND	ND	0.01±0.00	0.56±0.32
2399	Tetracosane§	0.06±0.03	0.27±0.38	0.01±0.00	0.52±0.17
2426	x-Methyltetracosane	ND	3.73±3.03	0.02±0.04	1.77±2.30
2500	Pentacosane§	6.33±0.72	3.31±3.39	0.07±0.04	1.14±0.10
2534	13-Methylpentacosane	7.17±3.4*	7.65±6.99*	0.03±0.01	ND
2539	7-Methylpentacosane	1.05±0.54	0.91±0.91	ND	ND
2552	5-Methylpentacosane§	0.55±0.65	2.57±4.68	0.23±0.03	2.91±0.94
2562	11.15-Dimethylpentacosane	0.65±0.38	5.24±3.37*	ND	ND
2570	3-Methylpentacosane§	2.87±1.25	2.40±2.30	0.03±0.05	1.39±1.55
2599	Hexacosane§	1.12±0.26	0.12±0.11	0.02±0.01	0.89±0.50
2633	12-Methylhesacosane	0.76±0.35	0.43±0.39	0.01±0.00	ND
2680	Heptacosene	0.56±0.46	0.15±0.21	0.01±0.01	ND
2692	Heptacosene	0.44±1.07	1.94±2.09	ND	ND
2700	Heptacosane§	13.09±3.63*	ND	1.12±0.27	2.29±1.02
2711	x-Methylheptacosane	ND	4.95±4.38*	0.02±0.04	1.1±0.69
2732	13-Methylheptacosane§	15.56±5.81*	9.74±9.41*	0.19±0.03	0.11±0.19
2742	7-Methylheptacosane	5.3±6.71	1.42±1.43	0.04±0.01	ND
2763	x-Methylheptacosane	1.41±0.79	0.47±0.89	ND	ND

Table 1. Percentage area of nonpolar compounds (> 0.5%) present in cuticle and venom of eusocial wasps *Apoica pallens* and *Polistes versicolor*. (Continuation)

Calculated index (kraftz)	Compounds	*Apoica pallens* Cuticle	*Apoica pallens* Venom	*Polistes versicolor* Cuticle	*Polistes versicolor* Venom
		Percentage (%±standard deviation)			
2765	x-Methylheptacosane	0.77±1.03	0.74±1.48	ND	ND
2770	3-Methylheptacosane§	10.83±1.99*	3.13±3.06	2.43±0.52	4.27±2.14*
2800	Octacosane§	0.48±0.08	0.13±0.16	0.3±0.06	1.66±1.60
2830	14-. 13-. 10-Methyloctacosane§	0.78±0.20	0.28±0.24	0.17±0.05	3.58±2.35
2845	6-Methyloctacosane	ND	4.67±4.84*	0.01±0.01	ND
2850	x-Methyloctacosane	ND	ND	0.03±0.04	0.57±0.51
2901	Nonacosane§	1.70±0.54	0.15±0.18	5.37±0.92*	3.04±2.10
2928	13-Methylnonacosene §	3.03±0.55	0.77±0.80	2.34±0.58	2.26±1.10
2933	15-Methylnonacosane	1.17±0.23	0.10±0.22	3.73±0.85*	ND
2936	13-Methylnonacosane	0.60±0.15	ND	0.22±0.04	ND
2941	7-Methylnonacosane	ND	0.03±0.07	0.61±0.17	0.10±0.18
2951	5-Methylnonacosane	ND	ND	0.55±0.08	0.18±0.31
2966	9.13-Dimethylnonacosane	ND	ND	0.98±0.33	0.04±0.08
2975	3-Methylnonacosane§	1.74±0.41	0.05±0.11	12.23±1.34*	4.16±2.23
2984	5.x-Dimethylnonacosane	ND	3.44±4.33	ND	0.15±0.26
3000	Triacontane§	0.11±0.10	0.07±0.17	0.19±0.04	1.81±1.67
3028	10-Methyltriacontane	0.22±0.05	ND	1.03±0.21	ND
3100	Hentriacontane§	0.08±0.03	1.17±2.51	1.17±0.34	1.07±0.95
3119	11-. 13-Methylhentriacontane	ND	ND	0.04±0.05	3.60±0.91
3128	13-Methylhentriacontane§	8.32±1.79*	0.80±0.84	26.07±3.45*	3.85±2.55
3137	9-+13-Methylhentriacontane	ND	ND	1.05±0.25	ND
3153	x-Methylhentriacontane	ND	ND	0.82±0.20	ND
3155	13.17-Dimethylhentriacontane	ND	ND	2.86±0.81	0.87±1.51
3165	7.15-Dimethylhentriacontane	ND	ND	0.87±0.24	ND
3160	11.19-Dimethylhentriacontane	2.22±0.52	ND	ND	ND
3174	5.15-Dimethylhentriacontane	ND	ND	1.56±0.7	ND
3202	Dotriacontane	0.10±0.06	ND	0.92±0.47	0.76±0.74
3228	16-. 14-Methyldocotriacontane	0.15±0.07	ND	1.85±0.32	ND
3240	x-Methyldotriacontane	ND	1.11±1.83	ND	ND
3284	x.y-Dimethyldotriacontane	0.84±1.18	ND	ND	ND
3329	15-Methyltritriacontane§	2.57±0.7	0.06±0.19	23.49±6.37*	2.23±0.17
3334	x-Methyltritriacontane	ND	ND	0.81±0.22	ND
3356	11.21-Dimethyltritriacontane	2.71±0.68	ND	ND	ND
3365	11.15-Dimethyltritriacontane (11.15-diMeC33	ND	0.55±0.99	0.43±0.12	ND
3401	Tetratriacontane	ND	ND	0.68±0.31	ND
3523	17-. 13-Methylpentatriacontane	ND	ND	0.75±0.11	ND
3745	13.23 Dimethylheptatriacontane	0.91±0.3	0.07±0.14	ND	ND

* = Major compounds; §= Compounds present in all samples; TR: trace (<0.005%); ND: not detected

The cuticle presented 13 unique compounds and the venom 26 (Table 1), and these two groups share 43 compounds.

In samples of *P. versicolor* cuticle 93 peaks were detected, 85 of these were identified (91.4%), with chain length ranging from C_{15} to C_{37}. The five major compounds were nonacosane (5.37%), 15-methylnonacosane (3.73%), 3-methylnonacosane (12.23%), 13-methylhentriacontane (26.07%) and 15-methyltritriacontane (23.49%) (Table 1). Branched alkanes were the most significant in number of compounds and abundance, representing 89% of compounds, followed by linear alkanes 10.4%, and alkenes 0.4% (Figs 1A and 2A).

In the venom of this species 64 peaks were detected

and 60 identified (93.8%), ranging from C_{15} to C_{33}. The major compounds (Table 1) were pentadecane (4.91%), hexadecane (4.74%), octadecane (11.75%), eicosene (4.33%) and 3-methylheptacosane (4.27%). Branched alkanes were the most significant regarding number of compounds and abundance, representing 46.3% of compounds, followed by linear alkanes 42.3%, and alkenes 7.5% (Figs 1B and 2B). In these samples, 10 are unique compounds of cuticle and 5 of venom (Table 1), and both share 49 compounds.

Among cuticular samples of both species, 41 compounds are shared, 25 are unique of *A. pallens* and 44 of *Polistes versicolor*. Among the samples of venom, 43 compounds are shared, 44 are unique of *A. pallens* and 17 unique of *P. versicolor*.

The discriminant analysis shows that there are significant differences between chemical composition of cuticle and venom of the two species (Fig 3), with Wilk's Lambda = 0.001, $p < 0.001$ and $F = 205.12$. In this analysis, the first canonical root explains 95% of the results.

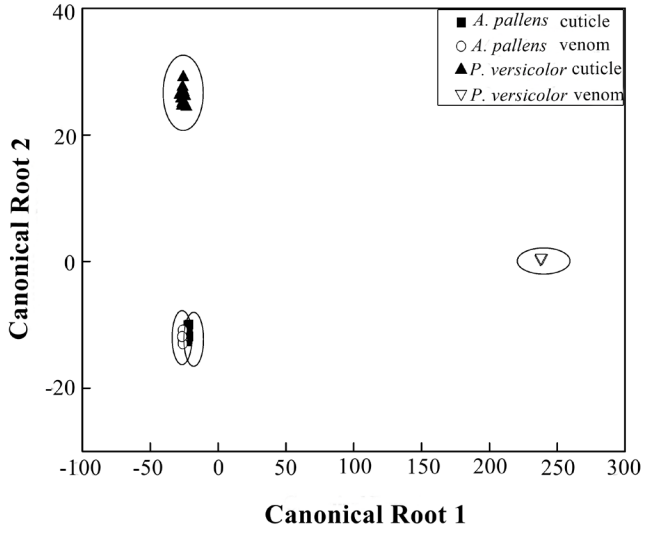

Fig 3. Dispersion diagram showing the differences in cuticular chemical profiles and chemical profile of nonpolar compounds of venom of *Apoica pallens* and *Polistes versicolor* wasps. Wilk's Lambda = 0.001, $p < 0.001$ and $F = 205.12$.

Discussion

The cuticle and venom of the two species differ in number and content of compounds, which was already expected (Bruschini et al., 2006b; Ferreira et al.,2012; Khidr et al., 2013; Soares et al., 2017) because they are different species. *P. versicolor* presented more compounds on the cuticle than in venom. However, in both species, both on the cuticle and in venom, the most important compounds in terms of abundance were the branched alkanes, moreover, the cuticle and venom of *P. versicolor* share more compounds than *A. pallens*.

In the cuticular chemical profile of *A. pallens*, the compounds range from C_{16} to C_{37}, and this variation in chain length of compounds from the cuticle is similar to those found for other species of Epiponini, such as *Polybia paulista* ranging from C_{19} to C_{36} (Kudô et al., 2017) and *Protopolybia exigua* ranging from C_{14} to C_{36} (Silva et al., 2016). In the cuticular profile of *P. versicolor*, the identified compounds range from C_{15} to C_{37}, differing from the *P. dominula*, whose compounds range from C_{21} to C_{35} (Lorenzi et al., 2004), and *P. fuscatus* ranging from C_{21} to C_{33} (Espelie et al., 1994). However, Brito et al. (2015) identified in the cuticular profile of the same species compounds that range from C_8 to C_{30}, although, in this study, the authors also evaluated cuticular compounds of immature stages.

The variation in chain length found in both species is relatively similar, thus, without considering the classes of compounds, the fact that one species builds populous colonies and the other small colonies, does not appear to have an influence on this aspect.

In the chemical profile of *A. pallens* venom, 87 nonpolar compounds were found ranging from C_{15} to C_{37}. However, in the venom of *P. exigua*, there is variation in chain length from C_{19} to C_{30} (Silva et al., 2016). Nonpolar compounds of *Polybioides raphigastra* venom vary from C_{11} to C_{18} (Sledge et al., 1999). In the venom of *P. versicolor*, 60 compounds were identified ranging from C_{15} to C_{37}. Bruschini et al. (2008) studying the volatile portion of venom from different castes of *P. dominula* found 42 compounds, but do not describe the chain length variation.

Samples of *P. versicolor* cuticle have 85 compounds and *A. pallens* 66. So, despite being an independent-founding species with relatively small colonies, its cuticular chemical composition seems to be more complex. Greater diversity in the profile of a species of *Polistes* wasp was described by Lorenzi et al. (2014), which associate the complexity in the cuticular profile of individuals from different colonies of *Polistes biglumis* to the presence of parasites.

The increase in complexity of cuticular profile makes it difficult for parasites to break the intracolonial code of wasps. This fact proves the adaptive plasticity of CHCs of wasps of the genus *Polistes* towards the influences in social environment (Lorenzi et al., 2014). It is likely that *P. versicolor* has greater susceptibility to parasitism because the occurrence of parasitism in this genus has been reported in several studies (Dapporto et al., 2007; Kudô et al., 2014; Lorenzi et al., 2014; Torres et al., 2016).

In both species studied, the branched alkanes are those that occur in greater abundance and number of compounds on the cuticle and in venom, followed by linear alkanes and finally alkenes (Figs 1 and 2). Similarly, Bonavita-Cougourdan et al. (1991) studying *Polistes dominula* also identified in the same order of abundance the compounds present on the cuticle of these wasps. It is known that branched alkanes seem to be more involved with signaling during intraspecific interactions (Lommelen et al., 2006). Dani et al. (2001) and Lorenzi et al. (2014) emphasize the communicative role of these compounds, since this class presents a high molecular

complexity, exhibiting high potential to encode information (LeConte & Hefetz, 2008; Blomquist & Bagnères, 2010). In this context, some authors consider branched alkanes the main mediators of chemical interactions between nestmates (Dani et al., 1996; Murakami et al., 2015).

Alkenes, although appearing in smaller proportions than other compounds in the samples of both species, also seem to be more related to the exchange of signals during chemical communication (Gibbs, 2002; Menzel et al., 2017). On the other hand, linear alkanes seem to be more involved in providing a barrier to prevent water loss (Armold & Regnier, 1975; Menzel et al., 2017), i.e. the impermeability of the cuticle. However, Tannure-Nascimento et al. (2007) studying *Polistes satan* identified greater abundance of linear alkanes in this wasp cuticle, suggesting that these compounds are also important as signals to mediate interactions between nestmates. The role of these compounds in venom, therefore, needs to be evaluated.

Both in the samples of *A. pallens* and *P. versicolor* there was higher concentration of heavy compounds on the cuticle, and light compounds in venom, varying significantly both qualitatively and quantitatively. According to Blomquist and Bagnères (2010) compounds below C_{20} are volatile and, therefore, can act as signals sent and received at a particular distance; while those of molecular weight above C_{20} can act as surface pheromone (Ishay et al., 1965).

The relatively lighter compounds, according to Blomquist and Bagnères (2010) are the most volatiles, and some studies with social wasps (Ishay et al., 1965; Jeanne, 1981; Sledge et al., 1999; Dani et al., 2000; Bruschini et al., 2006a, 2006b, 2008) demonstrated that colony defense is performed by a collective response of the volatile components of venom, which consequently act as alarm pheromones unleashing attacks and recruiting nestmates. Thus, the presence of a significant number of nonpolar compounds in the venom of both species suggests that at least part of them might be involved in some kind of chemical signaling. Indeed, Post and Jeanne (1984) assessed that volatile elements of the venom of three species of wasps are responsible for attracting and stimulating behavior of males, and that males of *P. fuscatus* respond to venom of *Polistes exclamans* and *Vespula maculifrons*, although the intensity of the response is smaller when compared to his own species, suggesting that at least some of the volatile components of venom are chemically similar and that these components can vary among species only in relative proportions.

More compounds were identified in the samples of *A. pallens* venom than in those of *P. versicolor*. It seems likely that *A. pallens* uses these compounds more effectively to mediate communication between nestmates, especially for colony defense, since by having large populations these wasps probably use volatile compounds of venom to alert nestmates, once even the nest structure is greater. On the other hand, as *P. versicolor* colonies contain few individuals, they may not use as effectively the compounds of venom for this purpose, or at least these compounds do not need to act at relatively long distances. In this context, greater complexity in venom profile might indicate greater action during interactions enabling that information reaches a greater number of workers.

P. versicolor shares qualitatively more compounds between samples of venom and cuticle than *A. pallens*. However, in Fig 3, the results show greater overlap of data of *A. pallens.* In this sense, the greater overlap is explained because samples of *A. pallens* display greater similarity in the content of shared compounds. Thus, for both species, the shared compounds suggest that they may be the most effective as signals exchanged during interactions between nestmates. Indeed, the literature has demonstrated the importance of variation in content of compounds for intraspecific chemical communication of wasps (Panek & Gamboa, 2000; Cotoneschi et al., 2009; Bonelli et al., 2015).

The greater overlap of data of *A. pallens* (Fig 3), suggest that the same compounds used to mediate interactions on the cuticle might also be used in venom. Therefore, due to being an evolutionary more derived species that is organized in more populous colonies, it may have a more elaborate signaling system. *P. versicolor* is an evolutionary less derived species whose colonies are relatively less populous and by this reason may have a more rudimentary alarm system (Jeanne, 1982).

In conclusion, this study showed that, despite being wasps of different foundation types that are organized in colonies with significantly different population number, the variation in chain length of compounds is relatively similar. In addition, in both types of samples of both species, the most important compounds are branched alkanes that are recognized as the most effective during interactions between nestmates. However, there is significantly greater similarity in content of shared compounds between samples of cuticle and venom of *A. pallens*, suggesting that the same compounds used to mediate interactions on the cuticle are also used in venom. Therefore, because it is a species that is organized in more populous colonies, it may have a more elaborate signaling system based on volatile compounds of venom, although, indeed, behavioral tests are needed to prove it.

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Authors' contribution

The original idea for this research was conceived by A Mendonça and WF Antonialli Junior. A Mendonça, KB Michelutti and CAL Cardoso performed the experiments and analyzed the data. A Mendonça drafted the manuscript. All the authors reviewed and approved the final manuscript.

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