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Variation of cuticular chemical compounds in three species of *Mischocyttarus* (Hymenoptera: Vespidae) eusocial wasps

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ABSTRACT

The social wasps have a remarkable system of organization in which chemical communication mediate different behavioral interactions. Among the compounds involved in this process, cuticular hydrocarbons are considered the main signals for nestmate recognition, caste differentiation, and fertility communication. The aims of this study were to describe the cuticular chemical compounds of the species *Mischocyttarus consimilis*, *Mischocyttarus bertonii*, and *Mischocyttarus latior*, and to test whether these chemical compounds could be used to evaluate differences and similarities between *Mischocyttarus* species, using gas chromatography coupled to mass spectrometry (GC–MS). Workers from these three species presented a variety of hydrocarbons ranging from C_{17} to C_{37}, and among the compounds identified, the most representative were branched alkanes, linear alkanes and alkenes. The results revealed quantitative and qualitative differences among the hydrocarbon profiles, as confirmed by discriminant analysis. This study supports the hypothesis that cuticular chemical profiles can be used as parameters to identify interspecific and intercolony differences in *Mischocyttarus*, highlighting the importance of these compounds for differentiation of species and populations.

Introduction

Social insects have a sophisticated colony organization system regulated mainly by chemical signals or pheromones, which are involved in all social activities (Wilson, 1965). Among these insects are the social wasps, which belong to the Vespidae family, divided in six subfamilies. Among these subfamilies is Polistinae, which englobes the genus *Mischocyttarus* Saussure (1853). This genus is the only representative of the tribe Mischocyttarini (Carpenter, 1993) and is the largest genus of social wasps, with more than 240 species distributed in nine subgenera (Carpenter and Wenzel, 1988; Silveira, 2008).

Their colonies are established by independent foundresses varying from one to a few queens (reproductive females) that start to build the nests (Jeanne, 1980; Von Ihering, 1896). A typical nest consists of a single comb attached to the substrate by a peduncle (Gadagkar, 1991; Jeanne, 1972; Wenzel, 1991). *Mischocyttarus* is an essentially Neotropical genus, with exception of a few species that occur in northern Mexico and Florida, USA (Hermann and Chao, 1983; Silveira, 1998, 2008), and has been considered of great importance in studies regarding the sociobiology (Jeanne, 1970, 1972; O'Donnell, 1999; Strassmann et al., 1995).

An important trait that plays a role in the cohesion of insect societies is the ability to distinguish between nestmates and non-nestmates (Singer et al., 1998). Chemical communication is very important for this purpose (Billen, 2006), as these insects use information provided by chemical compounds known as pheromones. Karlson and Luscher (1959) define pheromones as chemical signals produced by an organism that, even released in small quantities, may induce behavioral and/or physiological changes in another individual of the same species. These pheromones are generally divided into two types: light and volatile substances secreted by the exocrine glands and heavy hydrocarbon molecules found in the cuticle (Howard, 1993).

Cuticular hydrocarbons (CHCs) are compounds that essentially consist of carbon and hydrogen (Blomquist and Bagneres, 2010) and compose part of the lipid layer covering the cuticle of insects. They are essential to the survival of the individuals, because their

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primary function is to prevent dehydration (Lockey, 1988); at the same time, they form a protective barrier against microorganisms (Provost et al., 2008). The CHCs are synthesized by secreting cells derived from epidermal cells (Lockey, 1988) and are transported to the cuticle via hemolymph by lipophorin proteins (Bagneres and Blomquist, 2010).

Among the hundreds of CHCs identified in insects, there are three groups that stand out: n-alkanes, methyl branched alkanes, and unsaturated hydrocarbons (Blomquist, 2010). These compounds also act as signals during communication among insects, especially in social insects, enabling the recognition of conspecific individuals, nestmates, age, sex, and caste (Howard, 1993; Singer et al., 1998; Vander Meer and Morel, 1998). Variation can occur in the CHCs composition, depending on genetic and exogenous factors (Arnold et al., 1996; Blomquist and Bagneres, 2010; Dahbi et al., 1996; Gamboa et al., 1996; Kather and Martin, 2012; Page et al., 1991).

Another function of CHCs that has been previously explored in social wasps is their use as a complementary tool to assess variations among insect populations (Calderón-Fernández et al., 2005; Dapporto et al., 2004), as in the case of the species *Polistes dominula* (Christ, 1791) (Dapporto et al., 2004). Furthermore, previous studies have used the CHCs to differentiate species of termites (Kaib et al., 1991), ants (Martin et al., 2008), and Stenogastrinae wasps (Baracchi et al., 2010). Despite the recognized importance of these compounds as signaling or biochemical markers, there have been few studies with social wasps of the genus *Mischocyttarus*.

Mischocyttarus has been considered important for the study of sociobiology in wasps due to the incipient social organization and reproductive totipotence (Jeanne, 1970, 1972; Strassmann et al., 1995). However, the success of these studies depends on parallel efforts to rebuild relationships within this highly diverse genus (Silveira, 2008). Therefore, the aims of this study were 1) to describe the cuticular chemical compounds of the species *Mischocyttarus consimilis* (Zikan 1949), *Mischocyttarus bertonii* (Ducke, 1918), and *Mischocyttarus latior* (Fox, 1898), and 2) to test whether these chemical compounds could be used to evaluate differences and similarities between *Mischocyttarus* species.

Materials and methods

Collection

Twenty colonies were collected in different areas of the municipalities, two in Ivinhema (22°21′22.5″ S; 53°45′26.1″ W), 14 in Mundo Novo (23°56′23″ S; 54°17′25″ W), and three in Dourados (22°13′16″ S; 54°48′20″ W) in Mato Grosso do Sul State; and one in the municipality of Palotina (24°16′23″ S; 53°52′38″ W) in Paraná State, Brazil. Colonies of three species were collected: *Mischocyttarus (Kappa) bertonii*, *Mischocyttarus (Kappa) latior*, and *Mischocyttarus (Monocyttarus) consimilis*. All the adults were killed by freezing and stored until the moment of extraction of the compounds. Only workers were used, because differences between castes could influence the individual chemical composition. Castes were distinguished by behavioral observation prior to collection using the behavioral repertoire described by Torres et al. (2012) in *M. consimilis*. In addition, all colonies collected were in the post-emergence phase, according to the classification of Jeanne (1972).

Extraction of cuticular compounds for chemical analysis

The cuticular compounds were extracted by washing each individual for 2 min in 2 mL of hexane (Vetec, HPLC grade). The extracts were dried in an exhaustion chapel and stored until the day of the analysis, when the samples were solubilized in 200 μL of hexane. The CHCs were extracted from each female and the variation depended on the number of individuals in the colony, the colonies collected, and the species. The analyses were performed using a gas chromatograph coupled to a mass spectrometer (Model 2010 GCMS-QP, Shimadzu). A DB5-MS column was used (30.0 m length × 0.25 mm internal diameter, 0.25 μm film thickness), with heating from an initial temperature of 150 °C to 280 °C, at a rate of 3 °C/min, and maintaining the final temperature for 25 min. Helium (99.99%) was used as carrier gas, at a flow rate of 1 mL/min, and 1 μL sample volumes were injected in splitless mode. The temperatures of the injector, detector, and transfer line were 250 °C, 250 °C, and 290 °C, respectively. The mass spectrometer parameters included electron impact ionization voltage of 70 eV, mass range of 40–600*m/z*, and scan time of 0.3 s.

The cuticular compounds were identified using the retention indexes calculated using a series of linear alkanes (Van den Dool and Kratz, 1963), the library of the equipment (NIST21 and WILEY229), and analysis of the mass spectra. In the case of the linear alkanes (C_8–C_{40}), standards of the compounds were also used.

The peak area for each compound was determined by manual integration of the total ion chromatogram (TIC). All the areas were then transformed into relative percentage areas.

Statistical analysis

We used a discriminant analysis to separate the groups defined previously according to their chemical profiles of inter- and intraspecific differences. Wilks' Lambda was employed as a measure of the contribution of each variable. In these multivariate analyses, the percentage values were calculated from the chromatogram peak areas used as the independent variables.

Results

Interspecific variation among the three species of Polistinae wasps

The chemical compounds identified in the analyses of samples from the three species ranged from C_{17} to C_{37} (Table 1), corresponding to over 47% of the compounds detected and representing a relative proportion exceeding 76%. The three species presented 10 common compounds: 3-methyloctadecane, pentacosane, heptacosane, 3-methylheptacosane, octacosane, X-methyloctasane, 3-methyloctacosane, nonacosane, 13-methylnonacosane, and 3-methyltriacontane (Fig. 1). However, the use of relative proportions of these compounds in the chromatograms permitted the identification of quantitative differences between the species. For example, the relative percentage of 3-methyloctacosane was significantly higher in *M. latior* than in the two other species, and there were important contributions of 3-methylheptacosane and nonacosane in *M. bertonii* and *M. consimilis*, respectively (Fig. 1).

The compounds 6-methylpentacosane, 1 heptacosane, nonacosene, and 13,17-dimethyltriacontane only occurred in workers of the *M. bertonii* species. Workers of *M. latior* did not show any unique compounds (Table 1).

M. consimilis showed the highest number of detected (163) and identified (60) compounds, of which 13 were exclusive to this species: 4,8-dimethyloctacosane, 13,17-dimethylhentriacontane, 11-methyldotriacontane, 11,21-dimethyltritriacontane, 7-methyltritriacontane, X-methyltetratriacontane, X-methylpentatriacontane, 11,21-dimethylpentatriacontane, X,Y-dimethylpentatriacontane, 7,11-dimethylpentatriacontane, hexatriacontane, X-methylheptatriacontane and 11,21-dimethylheptatriacontane.

Table 1
Average relative proportions and their standard deviations for the cuticular hydrocarbons identified in three species of *Mischocyttarus* wasps.

Compound	Index calculated	*M. consimilis* Mean ± SD (%)	*M. bertonii* Mean ± SD (%)	*M. latior* Mean ± SD (%)
5-Methylheptadecane	1752	0.01 ± 0.10	0.03 ± 0.04	–
Octadecane	1800	0.13 ± 0.63	0.03 ± 0.02	–
3-Methyloctadecane	1872	0.15 ± 0.68	0.19 ± 0.08	0.09 ± 0.03
Nonadecane	1900	0.01 ± 0.70	–	0.06 ± 0.01
2-Methylnonadecane	1964	1.78 ± 0.70	0.59 ± 0.94	0.09 ± 0.12
Eicosane	2000	0.79 ± 0.27	0.55 ± 0.13	0.07 ± 0.04
Heneicosane	2100	0.07 ± 2.63	0.09 ± 0.13	0.03 ± 0.03
9-Methylheneicosane	2140	2.62 ± 2.58	3.23 ± 4.53	0.40 ± 0.72
2-Methylheneicosane	2170	6.04 ± 2.07	7.71 ± 3.45	–
Docosane	2200	0.35 ± 0.20	0.24 ± 0.15	–
2-Methyldocosane	2269	0.01 ± 0.16	–	0.12 ± 0.06
Tricosene	2272	0.02 ± 0.16	0.03 ± 0.07	–
Tricosane	2300	0.34 ± 0.16	0.89 ± 1.35	0.02 ± 0.01
X-methyltricosane	2329	0.05 ± 0.03	0.01 ± 0.01	–
7-Methyltricosane	2341	0.03 ± 0.03	0.01 ± 0.01	–
3-Methyltricosane	2373	0.03 ± 0.05	0.10 ± 0.12	–
Tetracosane	2400	0.03 ± 0.04	0.05 ± 0.09	0.01 ± 0.01
X,Y-dimethyltricosane	2407	0.01 ± 0.08	–	–
8-Methyltetracosane	2430	0.04 ± 0.16	0.17 ± 0.09	0.06 ± 0.03
10-Methyltetracosane	2431	0.03 ± 0.16	–	–
7-Pentacosene	2477	0.19 ± 0.17	0.19 ± 0.21	–
Pentacosane	2500	0.32 ± 0.17	2.27 ± 1.36	0.34 ± 0.12
X-methylpentacosane	2534	0.03 ± 0.09	0.01 ± 0.01	–
6-Methylpentacosane	2542	–	0.01 ± 0.01	–
5-Methylpentacosane	2549	0.22 ± 0.09	0.48 ± 0.23	0.38 ± 0.46
3-Methylpentacosane	2573	0.14 ± 0.06	0.67 ± 0.23	0.02 ± 0.02
Hexacosane	2600	0.06 ± 0.50	1.16 ± 1.82	0.18 ± 0.03
2-Methylhexacosane	2656	0.02 ± 0.50	–	–
3-Methylhexacosane	2672	0.06 ± 1.49	–	0.48 ± 0.08
1-Heptacosene	2676	–	27.95 ± 9.09	–
4,8-Dimethylhexacosane	2690	0.01 ± 1.47	–	–
Heptacosane	2700	1.61 ± 1.47	9.75 ± 1.33	11.48 ± 1.42
X,Y-dimethylheptacosane	2714	0.11 ± 1.43	1.35 ± 2.29	0.02 ± 0.05
X-methylheptacosane	2731	0.04 ± 1.43	0.53 ± 0.87	0.17 ± 0.10
7-Methylheptacosane	2741	0.01 ± 1.43	0.05 ± 0.06	–
5-Methylheptacosane	2750	0.11 ± 1.43	0.34 ± 0.55	0.06 ± 0.02
3-Methylheptacosane	2774	4.80 ± 1.44	7.80 ± 2.02	7.76 ± 0.67
Octacosane	2800	0.27 ± 0.27	0.61 ± 0.38	0.82 ± 0.17
3,11-Dimethylheptacosane	2810	0.04 ± 0.27	–	–
X-methyloctacosane	2828	0.55 ± 1.32	0.77 ± 0.83	0.55 ± 0.26
2-Methyloctacosane	2859	0.59 ± 1.33	0.58 ± 1.15	8.23 ± 16.32
3-Methyloctacosane	2874	0.22 ± 1.34	6.48 ± 4.57	34.5 ± 18.64
Nonacosene	2885	–	0.72 ± 0.79	–
4,8-Dimethyloctacosane	2890	0.11 ± 1.34	–	–
Nonacosane	2900	7.47 ± 1.32	3.88 ± 0.75	4.16 ± 2.34
13-Methylnonacosane	2934	1.26 ± 2.07	0.32 ± 0.23	2.92 ± 1.55
7-Methylnonacosane	2939	0.29 ± 2.14	–	–
5-Methylnonacosane	2952	0.15 ± 2.13	–	0.05 ± 0.04
X,Y-dimethylnonacosane	2956	0.29 ± 2.14	–	–
11,19-Dimethylnonacosane	2963	0.08 ± 2.13	–	–
7,17-Dimethylnonacosane	2970	0.81 ± 2.13	–	–
5,9-Dimethylnonacosane	2977	6.33 ± 2.09	2.29 ± 0.54	8.08 ± 0.69
Triacontane	3000	2.84 ± 1.40	0.32 ± 0.29	0.03 ± 0.03
14-Methyltriacontane	3034	1.56 ± 1.47	0.07 ± 0.08	0.02 ± 0.01
2-Methyltriacontane	3060	0.90 ± 2.64	–	0.04 ± 0.03
3-Methyltriacontane	3076	0.18 ± 2.66	–	2.12 ± 0.50
13,17-Dimethyltriacontane	3078	–	0.69 ± 0.43	–
Hentriacontane	3100	2.42 ± 2.58	3.25 ± 1.29	1.15 ± 0.40
X-methylhentriacontane	3135	4.33 ± 2.51	2.03 ± 1.07	0.25 ± 0.11
13,17-Dimethylhentriacontane	3157	7.27 ± 2.33	–	–
3-Methylhentriacontane	3178	1.44 ± 1.15	0.65 ± 0.67	–
Dotriacontane	3200	2.95 ± 1.12	0.22 ± 0.37	0.51 ± 0.11
11-Methyldotriacontane	3233	2.21 ± 0.82	–	–
12,16-Dimethyldotriacontane	3254	0.47 ± 0.34	0.18 ± 0.32	0.01 ± 0.01
2-Methyldotriacontane	3260	0.86 ± 0.44	–	–
Tritriacontane	3300	0.38 ± 0.68	0.38 ± 0.26	0.03 ± 0.06
13-Methyltritriacontane	3333	1.59 ± 1.06	1.46 ± 1.44	–
11,21-Dimethyltritriacontane	3339	0.29 ± 1.10	–	0.01 ± 0.01
7-Methyltritriacontane	3343	0.17 ± 1.09	–	–
13,17-Dimethyltritriacontane	3355	2.08 ± 1.09	0.24 ± 0.16	–
3-Methyltritriacontane	3379	0.56 ± 0.98	–	–
5,17-Dimethyltritriacontane	3387	0.66 ± 0.85	0.28 ± 0.39	0.05 ± 0.11
Tetratriacontane	3400	0.72 ± 0.68	0.01 ± 0.01	–

Table 1
(*Continued*)

Compound	Index calculated	*M. consimilis* Mean ± SD (%)	*M. bertonii* Mean ± SD (%)	*M. latior* Mean ± SD (%)
X-methyltetratriacontane	3421	0.05 ± 0.67	–	–
Pentatriacontane	3500	0.16 ± 0.46	0.01 ± 0.02	–
X-methylpentatriacontane	3529	0.65 ± 0.33	–	–
X,Y-dimethylpentatriacontane	3556	0.06 ± 0.28	0.04 ± 0.06	–
11,21-Dimethylpentatriacontane	3563	0.48 ± 0.29	–	–
X,Y-dimethylpentatriacontane	3556	0.05 ± 0.28	–	–
7,11-Dimethylpentatriacontane	3568	0.19 ± 0.28	–	–
Hexatriacontane	3600	0.34 ± 0.27	–	–
X-methylheptatriacontane	3721	0.06 ± 0.07	–	–
11,21-Dimethylheptatriacontane	3767	0.75 ± 0.56	–	–

SD, standard deviation.

In *M. consimilis*, the identified compounds represent a relative proportion of 76.72 ± 4.44%. In *M. bertonii*, 93 compounds were detected and 61 (64.5%) were identified, representing a relative proportion of 90.26 ± 4.71%. Finally, 81 compounds were detected in *M. latior* and from these 43 (53%) were identified, representing a relative proportion of 88.56 ± 3.07%. These findings confirmed qualitative differences among the different species, regarding cuticular compounds. For all the species, the compounds most frequently identified were the branched alkanes, linear alkanes, and alkenes (Fig. 2A and B). Notably, the presence of the alkene 1-heptacosene was only detected in *M. bertonii*, in higher relative proportion compared to other alkenes as well as other identified compounds (Fig. 2A and B).

Discriminant analysis showed significant quantitative differences in the relative proportions of the compounds common to the different species (Wilks' Lambda = 0.019, $F = 59.136$, $p < 0.001$; Fig. 3), and among the 11 compounds used in the analysis, 8 were important for separation of the species. The first and second canonical roots explained 61.2 and 27.4% of the results, respectively (total of 88.6%). The scatter plot (Fig. 3) shows clear separation among the species, indicating the existence of a typical cuticular chemical profile for each species.

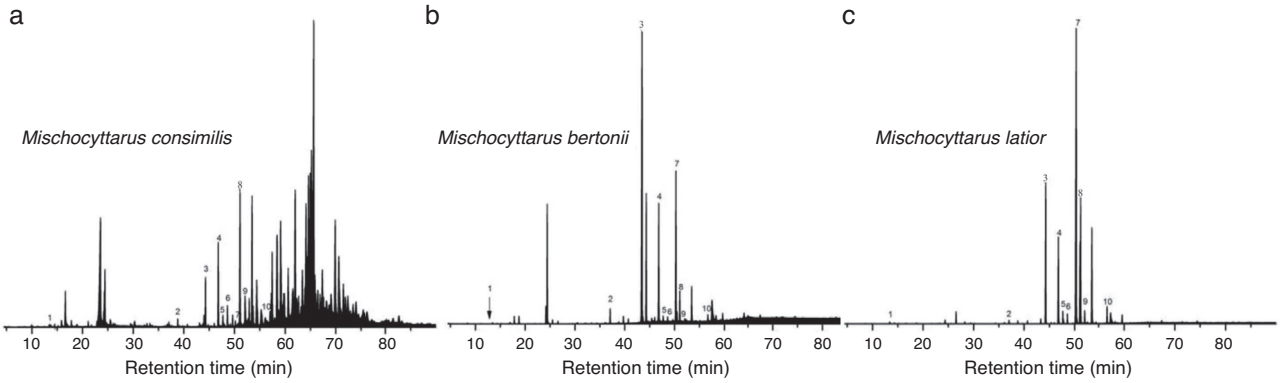

Fig. 1. Representative chromatograms for three species of social wasps of the genus *Mischocyttarus*, indicating the 10 compounds common to all of them. 1 = 3-methyloctadecane; 2 = pentacosane; 3 = heptacosane; 4 = 3-methylheptacosane; 5 = octacosane; 6 = X-methyloctacosane; 7 = 3-methyloctacosane; 8 = nonacosane; 9 = 13-methylnonacosane; 10 = 3-methyltriacontane.

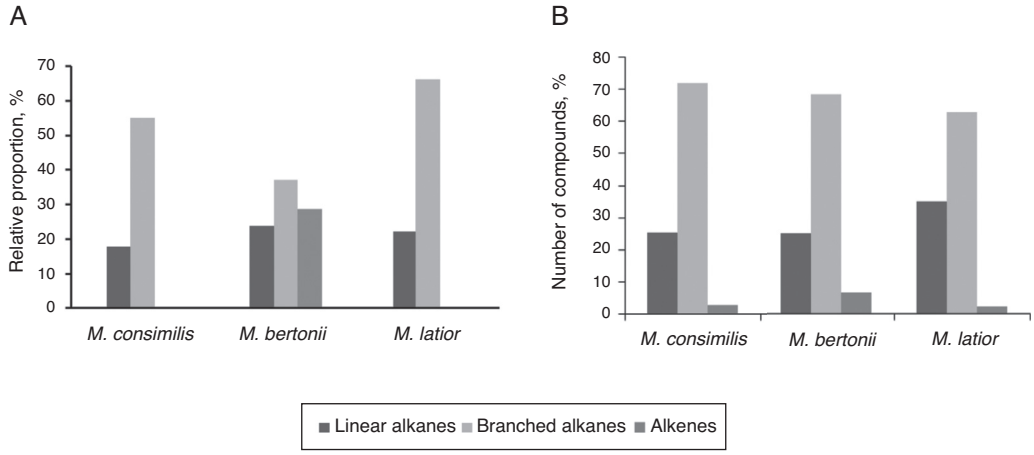

Fig. 2. Relative proportions (A) and numbers in percentage terms (B) of the compounds identified in the three social wasp species of the genus *Mischocyttarus*: *Mischocyttarus consimilis*, *Mischocyttarus bertonii*, and *Mischocyttarus latior*.

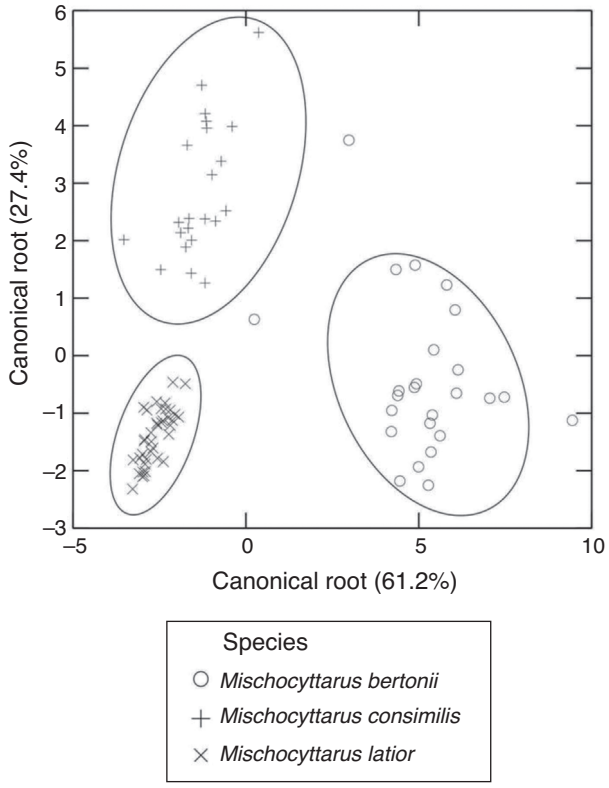

Fig. 3. Scatter diagram of the discriminant analysis results, showing the two canonical roots for differentiation of the three social wasp species of the genus *Michocyttarus*, based on the relative areas (percentages) of the cuticular compounds of females, obtained by GC–MS.

Intraspecific variation of cuticular chemical compounds among colonies of the three species of Mischocyttarus

In the case of *M. consimilis*, 44 of the 60 compounds identified (73.3%) were common to all colonies. Discriminant analysis revealed significant differences among the colonies (Wilks' Lambda = 0.001, $F = 2.426$, $p < 0.001$; Fig. 4), with 53 compounds being important for separation of the groups. The first and second canonical roots explained 80 and 8% of the results (total of 88%), as shown in Fig. 4.

For *M. bertonii*, only 21 of the 61 compounds identified (35%) were common to all colonies. The discriminant analysis also showed significant differences among colonies (Wilks' lambda = 0.001, $F = 6.647$, $p < 0.001$; Fig. 5), and out of the 19 compounds used, 13 were important for separation of the groups. The first and second canonical roots explained 83 and 9% of the results, respectively (Fig. 5).

Finally, of the 43 compounds identified in *M. latior*, 26 (60.4%) were common to all the colonies. Discriminant analysis showed significant differences among the colonies (Wilks' Lambda = 0.002, $F = 7.356$, $p < 0.001$; Fig. 6), with 9 of the compounds being important for separation of the groups. The first canonical root explained 63.3% of the results, and the second explained 34.4% (Fig. 6).

Discussion

The results indicate that the identified compounds comprised the greatest fraction (over of 76% of compounds) and are therefore likely to be the most important for the composition of the chemical signature. Among the identified compounds, the most abundant were branched alkanes, linear alkanes, and (to a lesser extent) alkenes (Fig. 2A and B). Branched alkanes were the most important compounds for interspecies separation (Fig. 3), evidencing the existence of a particular cuticular chemical profile for each species. Hence, the findings suggest that cuticular chemical profiles can be used as complementary tools to distinguish *Mischocyttarus* species.

Bagneres and Wicker-Thomas (2010) reported that CHCs could be used as chemotaxonomic parameters for species distinction. Studies using CHCs for distinction of social insect species were described by Kaib et al. (1991) for termites, Martin et al. (2008) for ants, and Baracchi et al. (2010) for hover wasps (Stenogastrinae), highlighting the importance of CHCs as a useful tool for species distinction.

Baracchi et al. (2010) suggested that the polar compounds present in the epicuticles of females of Stenogastrinae wasps

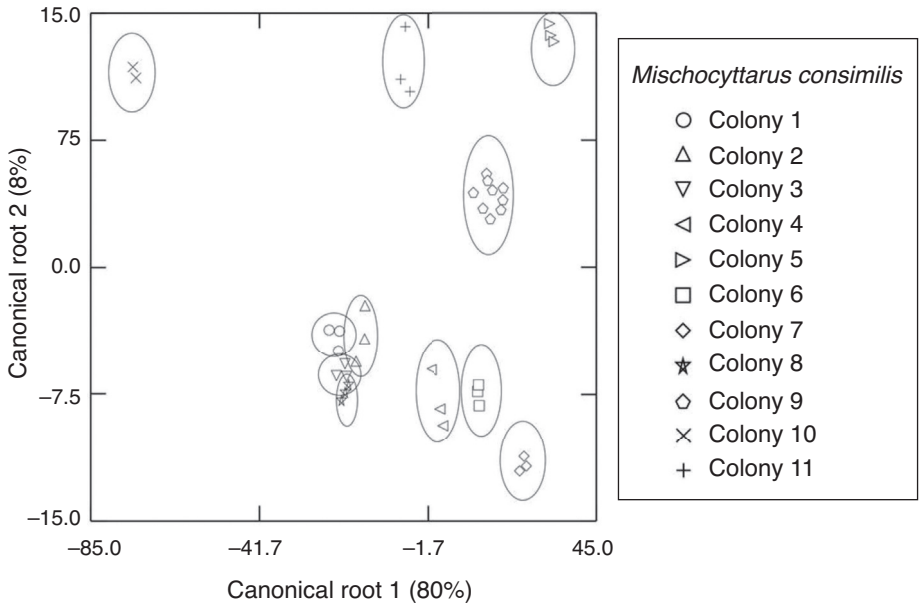

Fig. 4. Scatter diagram of the discriminant analysis results, showing the two canonical roots for differentiation of 11 colonies of *Mischocyttarus consimilis*, based on the relative areas (percentages) of the cuticular compounds of females, obtained by GC–MS.

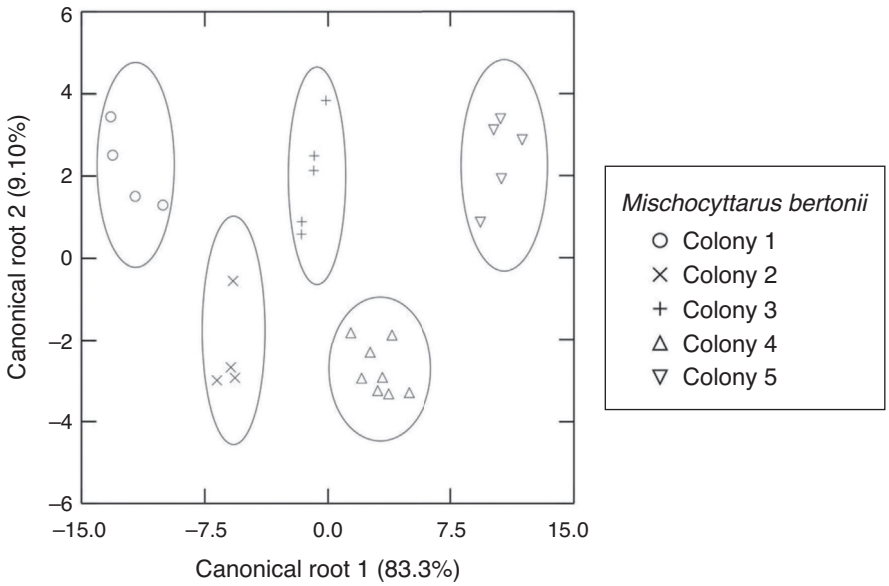

Fig. 5. Scatter diagram of the discriminant analysis results, showing the two canonical roots for differentiation of 5 colonies of *Mischocyttarus bertonii*, based on the relative areas (percentages) of the cuticular compounds of females, obtained by GC–MS.

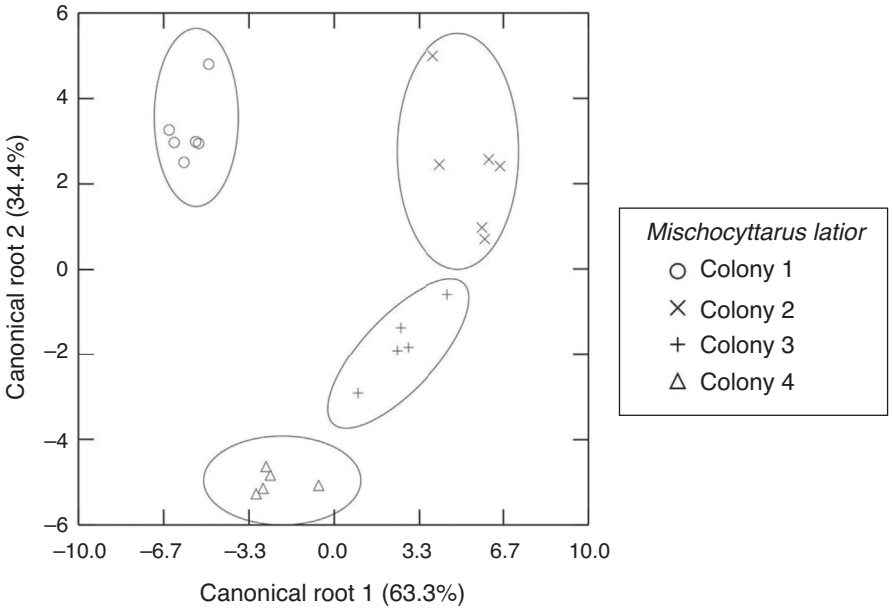

Fig. 6. Scatter diagram of the discriminant analysis results, showing the two canonical roots for differentiation of 4 colonies of *Mischocyttarus latior*, based on the relative areas (percentages) of the cuticular compounds of females, obtained by GC–MS.

could be used to identify different species, because the chemical parameters were consistent with the observed morphological characteristics. However, the authors point out that this tool is not always effective in distinguishing phylogenetically close species, as occurred in the species *Liostenogaster campanulae* (Turillazzi, 1990) and *Liostenogaster flavolineata* (Cameron, 1902). Kaib et al. (1991) analyzed the CHCs profiles of six species of *Odontotermes* (Holmgren, 1910) termites and concluded that intraspecific differences could be neglected when evaluating interspecific differences. Martin et al. (2008) investigated the chemical profiles of cuticular hydrocarbons in thirteen sympatric species of ants and concluded that the CHC profiles were stable, even considering ecological factors, with several species-specific hydrocarbons that could be used in taxonomic and evolutionary studies.

Ferreira et al. (2012), studying only the linear alkanes of three species of *Mischocyttarus*, identified 27 compounds of the same species. In the present study, in addition to the linear alkanes, 60 branched alkanes and 3 alkenes were also identified in *Mischocyttarus* species.

The use of these compounds as a taxonomic tool was previously proposed by Kather and Martin (2012), although it was suggested that chemotaxonomy might be more effective when used together with other traditional methods such as morphological, ecological, or molecular analysis. It was emphasized that the use of CHCs was reliable for this purpose, because these compounds are stable metabolic products that are hereditary and species-dependent.

Both qualitative and quantitative intracolonial differences were observed, and discriminant analysis confirmed significant

differences between colonies of *M. consimilis*, *M. bertonii*, and *M. latior*. Therefore, for these three species of *Mischocyttarus*, each colony had a specific cuticular chemical profile.

Several studies have described the importance of CHCs for intraspecific distinction (Bonavita-Cougourdan et al., 1991; Bruschini et al., 2010; Dani et al., 1996; Dapporto et al., 2004; Espelie et al., 1994; Layton et al., 1994; Lorenzi et al., 1997; Sledge et al., 2001). Sledge et al. (2001) and Tannure-Nascimento et al. (2007) found that significant differences in CHCs enabled distinction between colonies of *P. dominula* and *Polistes satan* (Bequard, 1940), respectively. The distinctive chemical profile of an individual colony is related to the fact that social insects are usually able to discriminate nestmates (Arnold et al., 2000; Bonavita-Cougourdan et al., 1987; Howard and Blomquist, 1982; Wilson, 1971), with the main chemical signals involved in this process being the CHCs (Espelie and Hermann, 1990; Howard and Blomquist, 2005).

The existence of an individual chemical profile for each colony (Figs. 4–6) supports the role of CHCs as contact pheromones for conspecific identification, corroborating previous results obtained by Bruschini et al. (2011), Monnin (2006) and Provost et al. (2008). It therefore appears that these compounds can act as chemical signals not only at the individual level, but also at the colony level (Antonialli-Junior et al., 2007; Cotoneschi et al., 2009; Dapporto et al., 2004, 2005; Izzo et al., 2010; Layton et al., 1994; Monnin, 2006; Neves et al., 2012; Sledge et al., 2001; Tannure-Nascimento et al., 2008).

For both interspecific and intraspecific distinction, branched alkanes were most important for separation of the groups, together with some linear alkanes. Other studies also found that branched alkanes were the most relevant components for conspecific discrimination in colonies of *P. dominula* (Bonavita-Cougourdan et al., 1991; Dani et al., 1996), *Polistes metricus* (Say, 1831) (Layton et al., 1994), and *Polistes biglumis bimaculatus* (Geoffroy, 1785) (Lorenzi et al., 1997).

The higher content of branched alkanes can be explained by the fact that the results presented here and in the study of Tannure-Nascimento et al. (2007) were derived from the analysis of adults, rather than immature individuals. Cotoneschi et al. (2007) evaluated different developmental stages of *P. dominula* and found a greater abundance of linear alkanes in brood and a greater abundance of branched alkanes in adults. This emphasizes the importance of branched alkanes as recognition signals in adults.

According to Brown et al. (1991), Cervo et al. (2008), Espelie and Hermann (1990) and Lorenzi et al. (2004) during progression from the egg stage to the adult there is a decrease in the abundance of linear alkanes and an increase in the content of branched alkanes. On the other hand, Gamboa et al. (1996) suggested that the presence of branched alkanes might increase the susceptibility of the insect to desiccation. However, the wide variety of branched alkanes suggests that these compounds have additional functions whose benefit outweigh the disadvantage of decreased waterproofing (Buckner, 1993).

The results showed that the cuticular chemical compounds varied significantly among the colonies, confirming the existence of a colonial signature based on cuticular hydrocarbons. Independently of species and colony, the most relevant compounds (in decreasing order of importance) were methyl alkanes, linear alkanes, dimethyl alkanes, and alkenes. The results confirm that cuticular chemical profiles can be used as complementary parameters to evaluate interspecific and intercolony differences in wasps of the genus *Mischocyttarus*. However, it is clear that future studies that involve a larger number of species and phylogenetically closer are still necessary to validate the method, since a tool of great utility would help to solve difficult cases of differentiation between related species.

Conflicts of interest

The authors declare no conflicts of interest

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