Phylogenetic Constraint Test in Cuticular Hydrocarbons of Neotropical Swarm-founding Social Wasps (Hymenoptera, Vespidae, Epiponini)

Dayana Alves da Silva Cunha (dayanaalvesdasilva@gmail.com)
UEMS: Universidade Estadual do Mato Grosso do Sul https://orcid.org/0000-0001-5833-3842
Rodolpho Santos Telles de Menezes
Universidade Federal de Santa Maria
Claudia Andrea Lima Cardoso
Universidade Estadual do Mato Grosso do Sul
William Fernando Antonialli Junior
Universidade Estadual do Mato Grosso do Sul

Research Article

Keywords: Chemical communication, Paper wasps, Phylogenetic signal, Sociability

Posted Date: May 2nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1591065/v1

Abstract

Cuticular hydrocarbons (CHCs) are present in several insects. One of the most important functions of these compounds in social insects is the exchange of signals during interactions between nestmates. Although we know about the functions performed by CHCs, we still have little information about how these compounds evolved within different groups of insects, especially among wasps. In this study, we examine the diversity and abundance of the cuticular hydrocarbon profile of 17 species of Epiponini wasps using a phylogenetic approach. We investigated phylogenetic constraints on the evolution of CHCs in Epiponini. We then calculated the phylogenetic signal for all compounds present in the studied species. For several CHC traits, the phylogenetic signal was low, indicating a random expectation. Moreover, within a phylogenetic context, we did not find a pattern of increasing or decreasing number of compounds or structural groups. However, we verified that Epiponini wasps, a tribe that shows colony foundation by swarming, exhibit a smaller number of CHC compounds than the tribes (Mischocyttarini and Polistini) that show an independent foundation. Probably this difference is related to their type of nest and nesting behavior.

Introduction

Cuticular hydrocarbons (CHCs) are found in the cuticular lipid layer of insects (Sprenger et al. 2018). These compounds are formed of hydrogen and carbon, showing great diversity in their composition, varying in chain size, branches, or double or triple bonds. The gradual elongation of fatty acids produces CHCs by malonate, unbranched or methyl malonate, and branched fractions (Leonhardt et al. 2016). These compounds allow the insect's cuticle to be viscous and fluid and, for this reason, function to prevent water loss and provide communication between insects by acting as chemical signals (Menzel et al. 2019; Holze et al. 2021).

The importance of CHCs in the process of chemical communication by social insects has been investigated throughout the past 40 years (Blum and Brand 1972; Lange et al. 1989; Neves et al. 2012; Kather and Martin, 2015; Menzel et al. 2017; Michelutti et al. 2018; Santos et al. 2018; Gomes et al. 2020; Menzel et al. 2019; Holze et al. 2021). These compounds can vary according to environmental and genetic factors (Kather and Martin 2015) (Thomas and Simmons 2008). Therefore, several studies have evaluated the role of CHCs as a complementary tool to assess biogeographic differences (Dapporto et al. 2004; Cunha et al. 2017), taxonomy, and phylogenetic relationships (Kather and Martin 2015).

The diversity of activities related to the exchange of chemical information among social insects includes the division of labor, the collaborative use of resources, and collective defensive actions, all reflected in the diversity of chemical signals required for the success of social insect colonies (Leonhardt et al. 2016). However, the increase in diversity in the chemical composition for information exchange does not necessarily correlate with greater signal complexity (Kather and Martin, 2015). Despite the high diversity of the chemical profile of CHCs in social insects, it is suggested that phylogenetically closely related species have a similar composition of CHCs (Kather and Martin 2012; Losos 2008; Menzel et al. 2017).

Phylogenetic constraints on CHC evolution can be investigated (Menzel et al. 2017, 2019). Using specific tools, it is possible to detect phylogenetic signals even from CHC profiles. With this information, it is possible to test two evolutionary scenarios for CHC traits. One scenario is based upon saltational evolution where radical changes in chemical traits are followed by phases of little or no evolutionary change (Menzel et al. 2017). Contrastingly, in a gradual evolution scenario, a distinct phylogenetic signal is detectable as a result of small changes in chemical composition (Menzel et al. 2017). In this context, it is possible to assess the presence of phylogenetic signals derived from CHC traits, comparing qualitative (Zimmermann et al. 2009; Dyer et al. 2014) and quantitative variations (Menzel et al. 2017).

Some studies have investigated patterns such as increases or decreases in the number of compounds and structural groups of CHCs in social insects (Kather and Matin 2015; Menzel et al. 2017). For example, when analyzing qualitative characters, Van Wilgenburg et al. (2011) detected that ants appeared to have changed the number of compounds or structural groups of CHCs during their evolutionary history. However, in some groups, it seems that it is not possible to

verify a pattern in the evolution of CHCs, for example, in different beetle species belonging to the genus *Dendroctonus*, *Ips* (Symonds and Elgar 2004) and *Crematogaster* ants (Menzel et al. 2017).

Regarding the CHC profile, qualitative and quantitative variation is probably related to the activation or deactivation of genes according to selective pressures (Steiner et al. 2007). According to Kather and Martin (2015), the silencing of specific genes for a long evolutionary period can explain intrageneric variations in the presence and absence of certain classes of CHCs. Furthermore, Kather and Martin (2015) analyzed the CHC profile of approximately 241 hymenopteran species and suggested that several CHC classes, and their associated biochemical pathways, were already present at the beginning of the diversification of these insects.

Epiponini (Hymenoptera: Vespidae) is formed by 19 genera distributed in the Neotropical region (Carpenter 2004; Menezes et al. 2020). These wasps exhibit interesting social characteristics such as cyclic oligogyny (variable number of functional queens), colony foundation by swarming, and well-defined age polyethism (West-Ebehard 1977; Noll 2013). These insects require an efficient chemical recognition system and, therefore, they are an interesting group in which to investigate evolutionary trends in the profile of their CHCs. However, little is known about the diversity and evolution of CHCs in this group. We have characterized the diversity and abundance of the cuticular chemical compound profile of 17 species of Epiponini wasps. Additionally, we have investigated whether the evolution of CHCs in Epiponini wasps is phylogenetically constrained. We calculated the phylogenetic signal for all compounds of CHC identified, as well as five CHC classes, using relative abundance in the analyzed species. We expect to detect a phylogenetic signal in the analyzed traits if they are phylogenetically constrained (see Van Wilgenburg et al. 2011; Menzel et al. 2017).

Material And Methods

Sampling.

We used an active search method to collect social wasp colonies of 17 species of Epiponini wasps, belonging to 11 genera in several localities of Brazil, representing a total of 116 workers from 38 colonies analyzed (for details see Table 1).

Table 1
Species and locality of collection of the Epiponini samples used in this study.

	Species	Number of colonies per location	Number of wasps per colony	Collection place	Coordinates
1	*Angiopolybia pallens* Lepeletier, 1836	2	9	Ilha de Itaparica – BA	13°00’59.08”S; 38°42’11.25”W
2	*Apoica pallens* Fabricius, 1804	1	2	Colatina – ES Ilha de Itaparica – BA	19°32’10.76”S; 40°37’47.68”W 13°00’59.08”S; 38°42’11.25”W
3	*Chartergus globiventris* Saussure, 1854	1	3	Nova Xavantina – MT Ribeirão Cascalheira – MT	14°40’29.21”S; 52°20’37.12”W 12°57’47.31”S; 51°49’29.37”W
4	*Clypearea* weyrauchi Richards, 1978	3	2	Iranduba – AM	3°16’47.83”S; 60°11’08.11”W
5	*Epipona media* Cooper, 2002	1	3	Ilhéus – BA Santa Terezinha – BA	14°47’53.26”S; 39°02’04.91”W 12°46’12.06”S; 39°31’28.40”W
6	*Leipomeles dorsata* Cooper, 2002	2	3	Ilhéus – BA	14°47’53.26”S; 39°02’04.91”W
7	*Metapolybia decorata* Gribodo, 1896	1	4	Ituberá – BA Pedra Branca – BA	13°44’07.51”S; 39°08’47.23”W 8°34’08.41”S; 39°27’17.63”W
8	*Metapolybia docilis* Richards, 1978	2	4	Pedra Branca – BA	8°34’08.41”S; 39°27’17.63”W
9	*Parachartergus fraternus* Gribodo, 1892	1	4	Rio Branco – AC Brasília – DF	9°58’28.56”S;67°48’35.34”W 15°47’51.06”S; 47°53’30.79”W
10	*Parachartergus pseudopicalis* Willinck, 1959	1	3	Itamaraju – BA Ilhéus – BA	17°02’14.13”S; 39°31’57.27”W 14°47’53.26”S; 39°02’04.91”W

	Species	Number of colonies per location	Number of wasps per colony	Collection place	Coordinates
11	*Polybia ignobilis* Haliday, 1836	1	5	Dourados – MS Fátima do Sul – MS	22º13’51.10”S; 54º48’47.07”W 22º22’34.61”S; 54º30’55.20”W
12	*Polybia occidentalis* Olivier, 1791	2	10	Dourados – MS Ilhéus – BA	22º13’51.10”S; 54º48’47.07”W 14º47’53.26”S; 39º02’04.91”W
13	*Polybia sericea* Olivier, 1792	1	2	Dourados – MS Ivinhema – MS Ilhéus – BA	22º13’51.10”S; 54º48’47.07”W 22º18’44.88”S; 53º49’35.37”W 14º47’53.26”S; 39º02’04.91”W
14	*Pseudopolybia vespiceps* de Saussure 1863	1	4	Manaus – AM Ribeirão Cascalheira – MT	3º07’33.09”S; 60º01’18.23”W 12º58’26.36”S; 51º49’29.34”W
15	*Synoeca chalibea* de Saussure 1852	2	3	Rio Branco – AC	9º58’28.56”S;67º48’35.34”W
16	*Synoeca ilheensis* Lopes & Menezes 2017	1	3	Ilhéus – BA Itamaraju – BA	14º47’53.26”S; 39º02’04.91”W 17º02’14.13”S; 39º31’57.27”W
17	*Synoeca surinama* Linnaeus 1767	1	2	Cruzeiro do Sul – AC Ilhéus – BA	7º38’14.40”S; 72º40’12.72”W 14º47’53.26”S; 39º02’04.91”W

Analysis of the Diversity and Abundance of Compounds in the Cuticular Profile of Hydrocarbons in Epiponini Wasps.

We extracted the cuticular hydrocarbons from workers by immersion in 2 mL of HPLC grade hexane for 5 minutes (Takematsu and Yamaoka 1997). We then removed the individuals from contact with the solvent and the extract was dried with an exhaust hood. Subsequently, we solubilized the extract in 400µL HPLC grade hexane.

Knowing that variation in the sizes of the different species analyzed can influence the profile of CHCs due to differences in the contact surface for the extraction of chemical compounds, we standardized our analyses based on the individual’s body mass. Thus, for relatively larger species (e.g. *Apoica pallens*, with a mass of approximately 0.105g per individual), CHCs were extracted from 2 workers for each analysis, while for relatively smaller species (e.g. *Polybia occidentalis*, with a mass of approximately 0.015g per individual), CHCs were extracted from 10 individuals, grouped for each analysis. With an average value of 0.230g ± 0.09g, we extracted the CHCs and recorded at least three readings from each colony for each species.

We used a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) with a mass detector (GC-MS 2010 Ultra) using a DB-5 fused silica capillary column (60 m in length x 0.25 mm internal diameter x 0.25 µm film thickness). The conditions for the analysis previously established were as follows: helium (99.999%) as carrier gas with a flow of 1.0 mL min^{-1}, with an injection volume of 1 µL in splitless mode; heating ramp with an initial temperature of 150ºC reaching 300ºC at 3ºC min^{-1}, and remaining at the final temperature for an average of 10 minutes. The injector temperature was 280°C, and the detector and the transfer line temperature was 300°C. The mass spectrometer scan parameters included an electron impact ionization voltage of 70 eV, in the mass range of 45 to 800 m/z, and a scanning interval of 0.5s.

To identify the chemical compounds, we analyzed the Retention Index (RI) using a mixture of linear alkanes (C7 - C40, Sigma - Aldrich with purity ≥ 98%) as a reference and compared them with indexes found in the literature (Broph et al. 1983; Scribe et al. 1990; Provost et al. 1994; Bonavita-Cougourdan et al. 1991; Takematsu and Yamaoka 1997; Johnson et al. 2001; Akino et al. 2002; Steinmetz et al. 2003; Priestap et al. 2003; Senatore et al. 2005; Morteza-Semnani et al. 2007; Smith et al. 2008; Yusuf et al. 2010; Weiss et al. 2014; Soares et al. 2017; Michelutti et al. 2018; Paula et al. 2018; Duarte et al. 2019). We associated the interpretation of the mass spectra obtained from the samples and compared with the databases (NIST21 and WILEY229).

The chromatograms were recorded using the Chrom Quest v5.0 program and analyzed using the GCMSsolution v2.5 software. We determined the peak area of each compound by manual integration of each total ion chromatogram (TIC). We then selected only the peaks identified as CHCs and transformed them into relative percentage areas to obtain the relative abundance of these compounds.

Statistical and Phylogenetic Signal Analysis.

To assess if there are shared and exclusive CHCs among the studied species, we applied an Individual Indicator Value (*IndVal*) analysis as proposed by Dufrêne and Legendre (1997). *IndVal* analyzes the association between the different compounds and the species analyzed, considering each compound independently, using relative abundance values. To determine which compounds were more representative within the group, we considered $P \leq 0.05$. To assess whether there is an evolutionary trend towards an increase in the number of compounds with more branches and chemical bonds between the classes of CHCs in Epiponini, we analyzed the presence of branched compounds with different chemical bonds, in addition to the presence of isomers of these CHCs.

To investigate if the CHCs are phylogenetically constrained in Epiponini, we used K statistics to detect the presence of a phylogenetic signal, defined as “the tendency of related species to resemble each other more than species taken randomly from the tree” (Blomberg et al. 2003). A K value less than 1 indicates low phylogenetic dependence, and vice versa (Blomberg et al. 2003). To accomplish this, we used an ultrametric phylogenomic tree for Epiponini as input (Menezes et al. 2020). We used Mesquite v3.6 software (Maddison and Maddison, 2018) to remove any terminal for which the CHC profile is unknown. We calculated the K value for all compounds present in the CHC profile of the species studied, and also for classes of CHC compounds identified, using the relative abundance of the compounds. We calculated K using the R platform v3.6.1 (Team 2017) with the *phytools* package, the *phylosig* command, and 1000 randomizations.

Results

The analysis of the cuticular profiles of the 17 species revealed a total of 37 different peaks, representing 41 CHC compounds (including co-eluting compounds), from five different classes, such as alkenes, alkadienes, *n*- alkanes, monomethylalkanes, and dimethylalkanes. Considering the *IndVal* statistic, 19 CHCs were more representative among all samples; they appear in several of the analyzed species ($P \leq 0.05$). We did not identify any unique compounds. In terms of relative abundance, the most representative CHC classes were monomethylalkanes, n-alkanes, and alkenes (Fig. 1; Tables 2 and 3), and they are present in all genera analyzed. The classes of compounds that stand out in numbers are *n*-alkanes and monomethylalkanes.

Table 3
Chemical diversity of the CHC profiles of 17 Epiponini species.

	Species	Number of compounds	Number and abundance (%) of class of HCs				
			Alkenes	Alkadienes	n-Alkanes	Monomethylalkanes	Dimethylalkanes
1	*Angiopolybia pallens*	39	4 (19%)	1 (2%)	13 (35%)	14 (39%)	7 (5%)
2	*Apoica pallens*	17	1 (9%)	1 (4%)	5 (19%)	6 (58%)	4 (10%)
3	*Chartergus globiventris*	14	3 (54%)	ND	5 (23%)	6 (23%)	ND
4	*Clypearea weyrauchi*	34	4 (33%)	ND*	12 (38%)	12 (28%)	5 (1%)
5	*Epipona media*	34	4 (36%)	1 (3%)	12 (25%)	11 (30%)	6 (6%)
6	*Leipomeles dorsata*	19	3 (44%)	ND	8 (34%)	8 (22%)	ND
7	*Metapolybia decorata*	31	3 (20%)	1 (3%)	11(18%)	10 (55%)	6 (4%)
8	*Metapolybia docilis*	27	3 (14%)	1 (3%)	12 (39%)	9 (40%)	2 (5%)
9	*Parachartergus fraternus*	27	3 (37%)	1 (3%)	8 (23%)	10 (31%)	5 (6%)
10	*Parachartergus pseudopicalis*	33	3 (27%)	1 (1%)	12 (29%)	11 (39%)	7 (4%)
11	*Polybia ignobilis*	17	2 (9%)	1 (5%)	5 (29%)	5 (47%)	4 (10%)
12	*Polybia occidentalis*	17	2 (6%)	1 (5%)	5 (24%)	5 (54%)	4 (11%)
13	*Polybia sericea*	16	1 (11%)	1 (5%)	5 (28%)	5 (47%)	4 (9%)
14	*Pseudopolybia vespiceps*	23	2 (20%)	ND	9 (65%)	9 (14%)	3 (1%)

* Compounds with an abundance ≤ 0.1 were not considered

	Species	Number of compounds	Number and abundance (%) of class of HCs				
15	*Synoeca chalibea*	22	2 (11%)	ND	10 (62%)	10 (27%)	ND*
16	*Synoeca ilhensis*	19	3 (44%)	ND	4 (37%)	8 (13%)	4 (6%)
17	*Synoeca surinama*	19	3 (39%)	ND	7 (42%)	9 (19%)	ND

* Compounds with an abundance ≤ 0.1 were not considered

The species with the highest number of compounds are *Angiopolybia pallens* (39 CHCs) and *Metapolybia decorata* (31 CHCs). Those with the minor number are *Apoica pallens* (17 CHCs), *Chartergus globiventris* (14 CHCs), and *Polybia sericea* (16 CHCs). We detected compounds with a methyl branch in all species, but with variations among the number of methyl compounds, with some species showing only five methyl compounds, up to 14 compounds, with methyl branchs (Tables 2 and 3). Furthermore, we detected methyl compounds in older lineages, such as *Apoica* and *Polybia*. Those with double bonds, such as alkenes, are present in all species. Alkadienes, although not present in all species, appear in groups such as *Polybia* (5%) and *Metapolybia* (2.53%), and in *Ap. pallens* (3.65%) (Table 2).

The K values obtained from the abundance of compounds from the five analyzed classes show that, in general, there is no phylogenetic signal (Table 4 and Supplementary information 1). However, when each separate compound was analyzed, we detected a phylogenetic signal in five compounds: 9-methylheptacosane (K = 1.13, P = 0.02), 5-methylnonacosane (K = 1.21, P = 0.01), 3,15-dimethylheptacosane (K = 1.02, P = 0.02), and 5.9 ; 5.11 - dimethylheptacosane (K = 1.08, P = 0.04) (Tables 4 and S1).

Table 4
Phylogenetic signal of cuticular hydrocarbon (CHC) traits in Epiponini wasps.

HC trait (quantitative) class	**K**	**P_{rand}**
Percentagem of alkenes	0.44	0.19
Percentagem of alkadienes	0.70	**0.05**
Percentagem of n-alkanes	0.50	0.23
Percentagem of methylalkanes	0.76	**0.02**
Percentagem of dimethylalkanes	0.31	0.38
HC trait (quantitative) compounds	**K**	**P_{rand}**
x-Methylheptacosane	**1.13**	**0.03**
5,9 and 5,11- Dimethylheptacosane	**1.08**	**0.04**
3,15-Dimethylheptacosane	**1.02**	**0.02**
5-Methylnonacosane	**1.21**	**0.01**

***** The table shows a K statistic (for all 5 quantitative traits grouped into classes) and only K values above 0.5 for all HC traits (quantitative) and the *p* value. Values in bold show phylogenetic signal.

Discussion

According to our results, the numbers of CHCs are in agreement considering the lowest number of CHCs found for Epiponini wasps, such as *Parachartergus aztecus* Willink 1959 with eight CHCs (Espelie and Hermann 1988), *Polybia micans* Ducke 1904 with 14 CHCs (Kelstrup et al. 2014a), *Polybia paulista* Haliday 1836 with 48 CHCs (Cunha et al. 2021), *Synoeca surinama* with 22 CHCs (Kelstrup et al. 2014b), and *Synoeca septentrionalis* (*ilheensis)* Lopes and Menezes 2017 with 11 CHCs (Santos et al. 2018).

Our results do not appear to show a pattern of increase or decrease in the number of compounds related to the phylogenetic information for the Epiponini species (Table 3). For example, *Angiopolybia*, a sister group to the other genera of Epiponini, and *Clypearia, Epipona*, and *Metapolybia*, which are more recent lineages (Menezes et al. 2020; Noll et al. 2021), have the highest numbers of CHCs in Epiponini (*Angiopolybia pallens* with 39 CHCs, *Clypearia weyrauchi*, *Epipona media*, and *Metapolybia decorata* with 31 CHCs each). Species with the lowest number of compounds are found in *Apoica* (*A. pallens* with 17 CHCs) and *Polybia* (*P. ignobilis* with 17 CHCs) (see Table 3).

In general, the number of compounds found in Epiponini wasps, a group that presents colony foundation by swarming, are smaller than those registered in wasps with independent foundation. For example, in studies with *Mischocyttarus consimilis* Zikán 1949, 79 CHCs were detected (Soares et al. 2017), in *M. cerberus styx* Richards 1940, 72 cuticular compounds were detected (Silva et al. 2020), and in *Polistes dominula* Christ 1791, 70 CHCs were detected (Beani et al. 2019). A higher and lower number of cuticular compounds in different groups of social wasps may be associated with the type of colony foundation (independent foundation *versus* swarm foundation). Colonies with an independent foundation are often more exposed to parasitism, both by parasitoids and social parasitism (Bagnères et al. 1996; Dapporto et al. 2004; Neves et al. 2013). Thus, the interaction of these species (parasite and host) in the same colony may have pressured species of independent colony foundation to invest in richer CHC profiles.

Considering Epiponini wasps, the same pattern is observed when different structural groups with double bonds and methyl groups are considered. According to the group's phylogenetic relationships, a pattern is not observed in either the presence or absence of these compounds or in an increase or decrease of numbers of a compound. However, analyzing the CHC profile in general, the number of species that produced these compounds is relatively small. When present, they appear with a low number of compounds, such as alkenes, or low abundance, such as alkadienes, compounds with double bonds (Table 3).

In Vespinae, a sister group of Polistinae (Menezes et al. 2020; Noll et al. 2021), many species have CHC profiles composed of *n*-alkanes, alkenes, monomethylalkanes, and dimethylalkanes (Van Zweden et al. 2014). In social wasps with independent foundation, for example, *Mischocyttarus* and *Polistes*, a sister group of Epiponini (Menezes et al. 2020), also present species with cuticular profiles composed of n-alkanes, mono-, di-, and trimethylalkanes, and alkenes, such as *M. consimilis*, *M. bertonii* Ducke 1918, and *M. latior* (Soares et al. 2017). However, alkenes are not present in *M. cassununga* (Murakami et al. 2015). In several *Polistes* species, all classes of compounds abovementioned are found, including trimethylalkanes (Elia et al. 2017; Keltrup et al. 2015; Oi et al. 2019; Murakami et al. 2015). On the other hand, some *Polistes* species do not have alkenes or trimethylalkanes (Dapporto et al. 2007). Furthermore, *Polistes versicolor* Olivier 1791 does not have alkenes, di-, or trimethylalkanes, but only n-alkanes and monomethylalkanes (Brito et al. 2015).

Considering groups with independent foundation of colonies such as Mischocyttarini and Polistini, the CHC profiles have n-alkanes and different methyl groups (Murakami et al. 2015; Soares et al. 2017; Oi et al. 2019). When comparing these insects with swarm founding wasps, there seems to be a tendency to "simplify" the CHC profile, considering the losses in some compounds and their abundance, as well as the simplification of the types of bonds and ramifications present in the compounds. Wasps with independent foundation behavior have a greater number of n-alkanes and methyl groups compared to Epiponini, which have more unsaturated compounds.

Furthermore, all the diversity of CHCs found among the analyzed Epiponini species is present in the cuticular profiles of *Angiopolybia* (*An. pallens*) and *Apoica* (*Ap. pallens*) (Fig. 1), genera with plesiomorphic features (Menezes et al. 2020; Noll et al. 2021), suggesting that these compounds could already be present in the Epiponini ancestor. When studying several hymenopteran, Kather and Martin (2015) indicated that all the compounds found were already present at the beginning of the group's evolutionary history.

Even if we compare our results with other hymenopterans, the number of compounds detected is similar to that described for some ant species, such as *Odontomachus bauri* Emery 1892 with 22 cuticular compounds, *Ectatomma brunneum* Smith 1858 with 23, *Atta sexdens* Forel 1980 with 24, *Formica argentea* Wheeler, 1912 with 28, and *Pachycondyla analysis* Latreille 1802 with 35 cuticular compounds (Yusuf et al. 2010; Krasnec and Breed 2013; Duarte et al. 2019).

In addition, the presence of olefins (alkenes and alkadienes) in social wasps is uncommon. The wasps have predominantly diversified their production of methyl-branched alkanes (Kather and Martin 2015), such as those found in the neotropical wasp *Mischocyttarus cassununga*, whose mono and dimethylalkanes are predominant in its CHC profile (Murakami et al. 2015), as detected in other wasps (Kather and Martin 2015). Thus, in addition to the similarity in the number of compounds, the presence of olefins in the Epiponini tribe is also a feature of the typical CHC profile of ants. For example, *Crematogaster* Lund 1831 ants also show the same classes of compounds found in Epiponini wasps (alkenes, alkadienes, alkanes, mono – methylalkanes) (Menzel et al. 2017). However, the authors also observed that alkenes, alkadienes, and dimethylalkanes did not occur together in the same *Crematogaster* species. In Epiponini, all analyzed species presented alkenes in the cuticle. Only six species (*C. globiventris, C. weyrauchi, L. dorsata, P. vespiceps, S. chalibea*, and *S. surinama*) did not show the dimethylalkanes compound. Only *Ap. pallens* and *Polybia* species presented the three classes together in their profile (Tables 2 and 3; Fig. 3).

The evolution of these compounds in Epiponini wasps probably occurred randomly, as we detected phylogenetic signals in only five compounds present in the cuticular profile of the studied species (Table 3). Therefore, the CHC profile in the group probably represents a mode of evolution closer to the saltational rather than the gradual mode of evolution. The phylogenetic signal analysis in Hymenoptera was also investigated in ants (Van Wilgenburg et al. 2011; Menzel et al. 2017), and a low phylogenetic signal was also detected in *Crematogaster* ants (Menzel et al. 2017), differing from what was proposed by Van Wilgenburg et al. (2011) who suggested a gradual evolution scenario for CHC profiles in ant species.

According to Menzel et al. (2017), only alkenes, alkadienes, and dimethylalkanes are phylogenetically informative, and monomethylalkanes are probably conserved in several Hymenoptera species. In our study, we detected a phylogenetic signal in two monomethylalkanes (9-methylheptacosane and 5-methylnonacosane) and three dimethylalkanes (5.9; 5.11 – dimethylheptacosane and 3,15-dimethylheptacosane) with a K value higher than 1, which reflects what was mentioned by Menzel et al. (2017) concerning the presence of monomethylalkanes.

The presence of a phylogenetic signal depends on the taxonomic level investigated (Van Wilgenburg et al. 2011; Kather and Martin 2015; Menzel et al. 2017). The difference in taxonomic scale may explain why CHC profiles may have random evolution if analyzed at the genus level as in the study by Menzel et al. (2017), or tribe, as in this study, while a contrasting pattern for gradual evolution can be found at a higher taxonomic genus level, as was found in a family-level study, as per Van Wilgenburg et al. (2011).

Conclusion

We did not find a strong phylogenetic signal among the CHCs of Epiponini species analyzed. We also did not find a pattern within the structural groups that accorded with the phylogenetic relationships of the group. However, it is possible to verify that species of this group that show foundation by swarming generally present a smaller number of compounds than wasps that show independent foundation (*Mischocyttarus* and *Polistes*), which is probably related to their type of nest and nesting behavior. We highlight that Epiponini wasps invest more in unsaturated compounds than wasps with independent

foundation, which invest in compounds with more methyl groups. These differences may reflect the different selective pressures that act on CHCs, which are still poorly understood and, therefore, more studies are needed to better understand how these compounds evolved in this group of insects.

Declarations

ACKNOWLEDGMENT

We thank Dr. Yzel Rondon Súarez for assistance with statistical analysis. This study was partially funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Braszil (CAPES) – funding code 001 (concession number 88887.484350/2020-00 DASC). In addition to Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT), Financiadora de Inovação e Pesquisas (FINEP), Programa Institucional de Bolsas aos Alunos de Pós-Graduação (PIBAP/UEMS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (concession number 311975/2018-6 CALC) (concession number 308182-/2019-7 WFAJ) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (RSTM nº 2015/02432-0 and nº 2016/21098-7). We also thank two anonymous reviewers for their valuable suggestions and comments. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Cunha, D. A. S. = conceived and designed the study; sample collection; collected and analysed the data; drafted the initial version of the manuscript and all authors contributed to later versions of the manuscript. Menezes, R. S. T = conceived and designed the study; sample collection; collected and analysed the data; drafted the initial version of the manuscript and all authors contributed to later versions of the manuscript. Cardoso, C. A. L. = collected and analysed the data; contributed to later versions of the manuscript. Antonialli-Junior, W. F. = conceived and designed the study; drafted the initial version of the manuscript and all authors contributed to later versions of the manuscript.

References

1. Akino T, Terayama M, Wakamura S, Yamaoka R (2002) Intraspecific Variation of Cuticular Hydrocarbon Composition in *Formica japonica* Motschoulsky (Hymenoptera: Formicidae). Zool Sci 19:1155–1165
2. Bagnères AG, Lorenzi MC, Dusticier G, Turillazzi S, Clement JL (1996) Chemical Usurpation of a Nest by Paper Wasp Parasites. Sci 272:889–892
3. Beani L, Bagnères AG, Elia M, Petrocelli I, Cappa F, Lorenzi M (2019) Cuticular hydrocarbons as cues of sex and health condition in *Polistes dominula* wasps. Insectes Soc 66(4):543–553
4. Blomberg SP, Garland TJ, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evol 57(4):717–745
5. Blum MG, Brand JM (1972) Social Insect Pheromones: Their Chemistry and Function. Am Zool 12(3):553–576
6. Bonavita-Cougourdan A, Theraulaz G, Bagnéres AG, Roux M, Pratte M, Provost E et al (1991) Cuticular hydrocarbons, social organization and ovarian development in a polistine wasp: *Polistes dominulus* christ. Comp Biochem Physiol 100B(4):667–680
7. Brito JH, Montagna TS, Maia FS, Antonialli-Junior WF, Cardoso CAL (2015) Cuticular signature in the development of *Polistes versicolor*. Gen Mol Res 14(4):12520–12528
8. Brophy JJ, Cavill GWK, Davies NW, Gilbert TD, Philp RP, Plant WD (1983) Hydrocarbon constituents of three species of Dolichoderine ants. Insect Biochem 13:381–389
9. Carpenter JM (2004) Synonymy of the genus Marimbonda Richards, 1978, with *Leipomeles moebius*, 1856 (Hymenoptera: Vespidae: Polistinae), and a new key to the genera of paper wasps of the new world. Am Mus Novit 3456:1–16

10. Cunha DAS, Menezes RST, Costa MA, Lima SA, Andrade LHC, Antonialli-Junior WF (2017) Integrated Analyses of Cuticular Hydrocarbons, Chromosome and mtDNA in the Neotropical Social Wasp *Mischocyttarus consimilis* Zikán (Hymenoptera, Vespidae). Neotrop Entomol 46(6):642–648
11. Cunha DAS, Menezes RST, Cardoso CAL, Antonialli-Junior WF (2021) Is It Possible to Obtain the Chemical Profile From Ethanol-Preserved Specimens? The Hydrocarbon and Fatty Acid Composition of the Social Wasp *Polybia paulista* (Hymenoptera: Vespidae: Epiponini). Environ Entomol 50(3):580–588
12. Dapporto L, Palagi E, Turillazzi S (2004) Cuticular hydrocarbons of *Polistes dominulus* a biogeographic tool: a study of populations from the Tuscan archipelago and surrounding areas. J Chem Ecol 30:2139–2151
13. Dapporto L, Dani FR, Turillazzi S (2007) Social dominance molds cuticular and egg chemical blends in a paper wasp. Cur Biol 17:r504–r505
14. Duarte BF, Michelutti KB, Antonialli-Junior WF, Cardoso CAL (2019) Effect of temperature on survival and cuticular composition of three different ant species Effect of temperature on survival and cuticular composition of three different ant species. J Therm Biol 80:178–189
15. Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for flexible asymmetrical approach. Ecol Monogr 67(3):345–366
16. Dyer KA, White BE, Sztepanacz JL, Bewick ER, Rundle HD (2014) Reproductive character displacement of epicuticular compounds and their contribution to mate choice in *Drosophila subquinaria* and *Drosophila recens*. Evolution 68:1163–1175
17. Espelie KE, Hermann HR (1988) Congruent cuticular hydrocarbons: biochemical convergence of a social wasp, an ant and a host plant. Biochem Syst Ecol 16(5):505–508
18. Gomes B, Lima CS, Silva M, Noll FB (2020) High Number of Species of Social Wasps (Hymenoptera, Vespidae, Polistinae) Corroborates the Great Biodiversity of Western Amazon: a Survey from Rondônia, Brazil. Sociobiology 67(1):112–120
19. Holze H, Schrader L, Buellesbach J (2021) Advances in decifrar a base genética da biossíntese e variação de hidrocarbonetos cuticulares de insetos. Heredity 126:219–234
20. Johnson CA, Vander Meer RK, Lavine B (2001) Changes in the cuticular hydrocarbon profile of the slave-maker ant queen, *Polyergus breviceps* emery, after killing a Formica host queen (Hymenoptera: Formicidae). J Chem Ecol 27:787–1804
21. Kather R, Martin SJ (2012) Cuticular hydrocarbon profiles as a taxonomic tool: advantages, limitations and technical aspects. Physiol Entomol 37:25–32
22. Kather R, Martin SJ (2015) Evolution of cuticular hydrocarbons in the Hymenoptera: a meta-analysis. J Chem Ecol 41:871–883
23. Kelstrup HC, Hartfelder K, Nascimento FS, Riddiford LM (2014a) Reproductive status, endocrine physiology and chemical signaling in the Neotropical, swarm-founding eusocial wasp *Polybia micans*. J Exp Biol 217:2399–2410
24. Kelstrup HC, Hartfelder K, Nascimento FS, Riddiford LM (2014b) The role of juvenile hormone in dominance behavior, reproduction and cuticular pheromone signaling in the caste-flexible epiponine wasp, *Synoeca surinama*. Front Zool 11(78):1–19
25. Krasnec MO, Breed MD (2013) Colony-Specific cuticular hydrocarbon profile in *Formica argentea* ants. J Chem Ecol 39:59–66
26. Lange C, Basselier JJ, Bagneres AG, Escoubas P, Lemaire M, Lenoir A et al (1989) Strategy for the analysis of cuticular hydrocarbons waxes from insects using gas chromatography/mass spectrometry with electron impact and chemical ionization. Biomed Environ Mass Spectrom 18:787–800
27. Leonhardt SD, Menzel F, Nehring V, Schmitt T (2016) Ecology and Evolution of Communication in Social Insects. Cell 164(6):1277–1287

28. Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol lett 11(10):995–1007
29. Maddison WP, Maddison DR (2018) Mesquite: A modular system for evolutionary analysis. Version 3.40. 2018. Available at: [http://mesquiteproject.org]
30. Menezes RST, Lloyd MW, Brady SG (2020) Phylogenomics indicates Amazonia as the major source of Neotropical swarm-founding social wasp diversity. Proc Royal Soc B: Biol Sci 287:20200480
31. Menzel F, Blaimer BB, Schmitt T (2017) How do cuticular hydrocarbons evolve? Physiological constraints and climatic and biotic selection pressures act on a complex functional trait. Proc Royal Soc B: Biol Sci 284(1850):20161727
32. Menzel F, Morsbach S, Martens JH, Räder P, Hadjaje S, Marinho P et al (2019) Communication vs. waterproofing: the physics of insect cuticular hydrocarbons. *J Exp Biol* 222(23): jeb.210807
33. Michelutti KB, Soares ERP, Sguarizi-Antonio D, Piva RC, Súarez YR, Cardoso CAL et al (2018) Influence of temperature on survival and cuticular chemical profile of social wasps. J Therm Biol 71:221–231
34. Morteza-Semnani K, Saeedi M, Akbarzadeh M (2007) Essential oil composition of *Teucrium scordium*. L Acta Pharm 57:499–504
35. Murakami ASN, Nunes TM, Desuó IC, Shima SN, Mateus S (2015) The Cuticular Hydrocarbons Profiles in the Colonial Recognition of the Neotropical Eusocial Wasp, *Mischocyttarus cassununga* (Hymenoptera: Vespidae). Sociobiology 62(1):109–115
36. Neves EF, Andrade LHC, Súarez YR, Lima SM, Antonialli-Junior WF (2012) Age-related changes in the surface pheromones of the wasp *Mischocyttarus consimilis* (Hymenoptera: Vespidae). Genet Mol Res 11(3):1891–1898
37. Neves EF, Montagna TS, Andrade LHC, Suarez YR, Lima SM, Antonialli-Junior WF (2013) Social Parasitism and Dynamics of Cuticular Hydrocarbons in Paper Wasps of the Genus *Mischocyttarus*. J Kans Entomol Soc 86:69–77
38. Noll FB (2013) "Marimbondos": a review on the neotropical swarm-founding polistines. Sociobiology 60(4):347–354
39. Carpenter JM (2021) Marimbondos: systematics, biogeography, and evolution of social behaviour of neotropical swarm-founding wasps (Hymenoptera: Vespidae: Epiponini). Cladistics 37(4):423–441
40. Oi CA, Oliveira RC, Van Zweden JS, Mateus S, Millar JG, Nascimento FS et al (2019) Do Primitively Eusocial Wasps Use Queen Pheromones to Regulate Reproduction? A Case Study of the Paper Wasp *Polistes satan*. Front Ecol Evol 7(199):1–10
41. Paula MC, Michelutti KB, Eulalio ADMM, Piva RC, Cardoso CAL, Antonialli-Junior WF (2018) New method for estimating the post-mortem interval using the chemical composition of different generations of empty puparia: Indoor cases. PLoS ONE 13:e0209776
42. Priestap HA, Van Baren CM, Di Leo Lira P, Coussio JD, Bandoni AL (2003) Volatile constituents of *Aristolochia argentina*. Phytochem 63:221–225
43. Provost E, Riviere G, Roux M, Bagnères AG, Clement JL (1994) Cuticular hydrocarbons whereby Messor barbarus ant workers putatively discriminate between monogynous and polygynous colonies. Are workers labeled by queens? J Chem Ecol 20:2985–3003
44. R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
45. Reeve HK, Sherman PW (2001) Optimality and phylogeny: a critique of current thought. p. 45–63 In.: Orzack, S. H.; Sober, E. eds. Adaptationism and optimality. Cambridge Univ. Press, Cambridge, U.K. 2001
46. Santos EB, Shemilt S, Carvalho CAL, Martin SJ (2018) Life history and chemical ecology of the Warrior wasp *Synoeca septentrionalis* (Hymenoptera: Vespidae, Epiponini). PLoS ONE 13(3):e0194689
47. Scribe P, Ngoumbi-Nzouzi JS, Fuché C, Pèpe C, Saliot A (1990) Biogeochemistry of organic matter in Lake Geneva: I. Particulate hydrocarbons as biogenic and anthropogenic molecular markers. Hydrobiol 207:319–331

48. Senatore F, Arnold NA, Bruno M (2005) Volatile components of *Centaurea eryngioides* Lam. and *Centaurea liberica* Trev. var. *hermonis* Boiss. Lam., two Asteraceae growing wild in Lebanon. *Nat Prod Res* 19:749–754
49. Silva RC, Silva AP, Assis DS, Nascimento FS (2019) The First Record of the Parasitoid Wasp Pachysomoides, Associated with *Mischocyttarus cerberus* Nests and Some Insights About the Parasitoid Wasp's Behavior. *Rev bras zoociências* v. 20, n. 1, p. 1–7, 2019
50. Smith AA, Hölldobler B, Liebig J (2008) Hydrocarbon signals explain the pattern of worker and egg policing in the ant *Aphaenogaster cockerelli*. J Chem Ecol 34:1275–1282
51. Soares ERP, Batista NR, Souza RS, Torres VO, Cardoso CAL, Nascimento FS et al (2017) Variation of cuticular chemical compounds in three species of *Mischocyttarus* (Hymenoptera: Vespidae) eusocial wasps. Rev Bras Entomol 61(3):224–231
52. Sprenger PP, Menzel F (2020) Cuticular hydrocarbons in ants (Hymenoptera: Formicidae) and other insects: how and why they differ among individuals, colonies, and species. Myrmecol News 30:1–26
53. Sprenger PP, Burkert LH, Abou B, Federle W, Menzel F (2018) Coping with the climate: cuticular hydrocarbon acclimation of ants under constant and fluctuating conditions. J Exp Biol 221(9):jeb171488
54. Steiner S, Mumm R, Ruther J (2007) Courtship pheromones in parasitic wasps: Comparison of bioactive and inactive hydrocarbon profiles by multivariate statistical methods. J Chem Ecol 33:825–838
55. Steinmetz I, Schmolz E, Ruther J (2003) Cuticular lipids as trail pheromone in a social wasp. Proc Royal Soc B: Biol Sci 270(1513):385–391
56. Symonds MRE, Elgar MA (2004) The mode of pheromone evolution: evidence from bark beetles. Proc Royal Soc B: Biol Sci 271:839–846
57. Takematsu Y, Yamaoka R (1997) Taxonomy of Glyptotermes (Isoptera, Kalotermitidae) in Japan with reference to cuticular hydrocarbon analysis as chemotaxonomic characters. ESAKIA 37:1–14
58. Thomas ML, Simmons LW (2008) Cuticular hydrocarbons are heritable in the cricket *Teleogryllus oceanicus*. J Evol Biol 21:801–806
59. Van Wilgenburg E, Symonds MRE, Elgar MA (2011) Evolution of cuticular hydrocarbon diversity in ants. J Evol Biol 24(6):1188–1198
60. Van Zweden JS, Bonckaert W, Wenseleers T, d'Ettorre P Queen signaling in social wasps.Evolution68(4):976–986
61. Weiss K, Parzefall C, Herzner G (2014) Multifaceted defense against antagonistic microbes in developing offspring of the parasitoid wasp *Ampulex compressa* (Hymenoptera, Ampulicidae). PLoS ONE 9:e98784
62. West-Eberhard MJ (1977) The establishment of reproductive dominance in social wasp colonies. Proc. 8Th Int. Cong. Int. Union Study Soc. Insects 223–227
63. Yusuf AA, Pirk CWW, Crewe RM, Njagi PGN, Gordon I, Torto B (2010) Nestmate recognition and the role of cuticular hydrocarbons in the african termite raiding ant *Pachycondyla analis*. J Chem Ecol 36:441–448
64. Zimmermann Y, Ramírez SR, Eltz T (2009) Chemical niche differentiation among sympatric species of orchid bees. Ecology 90:2994–3008

Table 2

Table 2 is available in the Supplementary Files section.

Figures

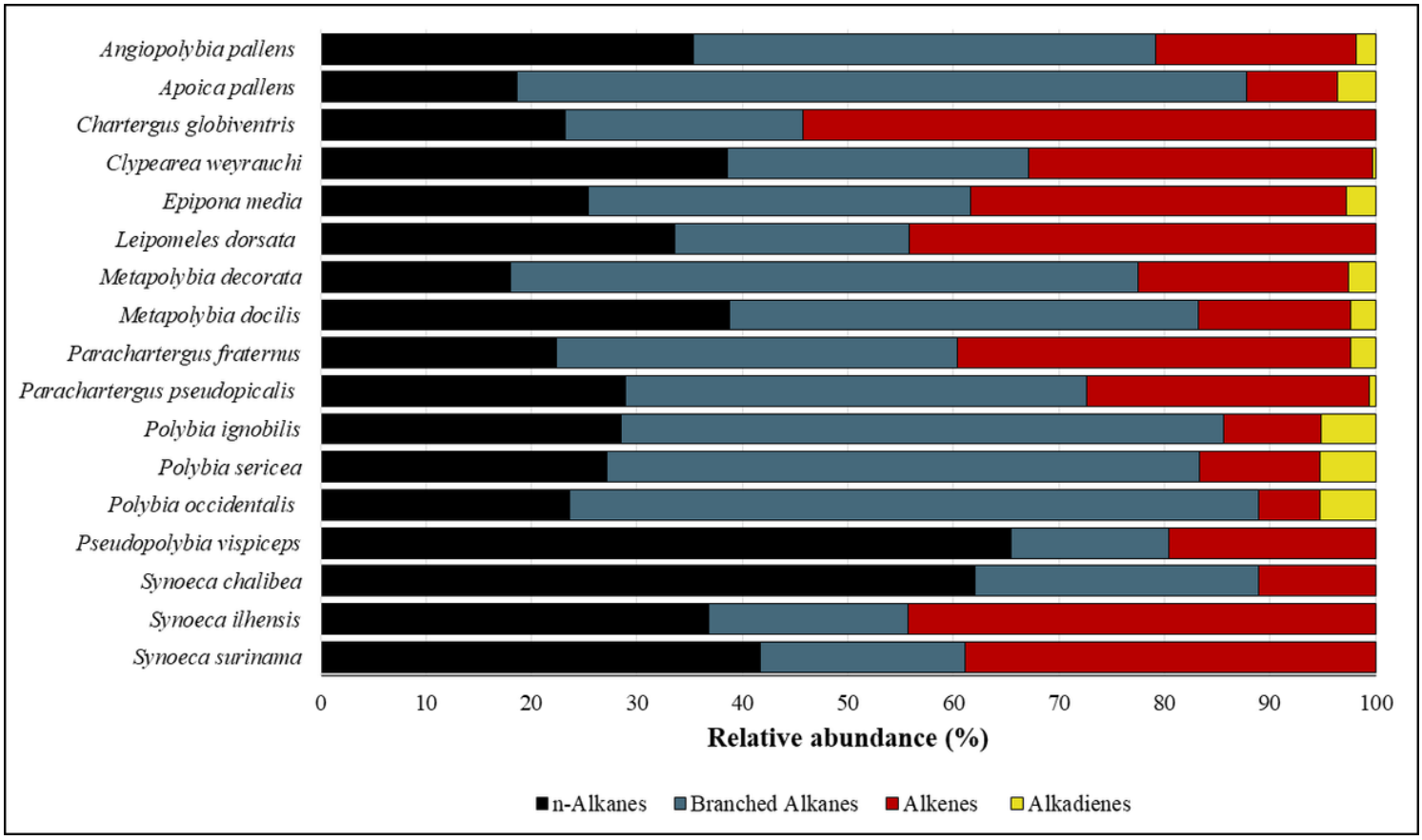

Figure 1

Percentage of compounds, using relative abundance, present in Epiponini species.

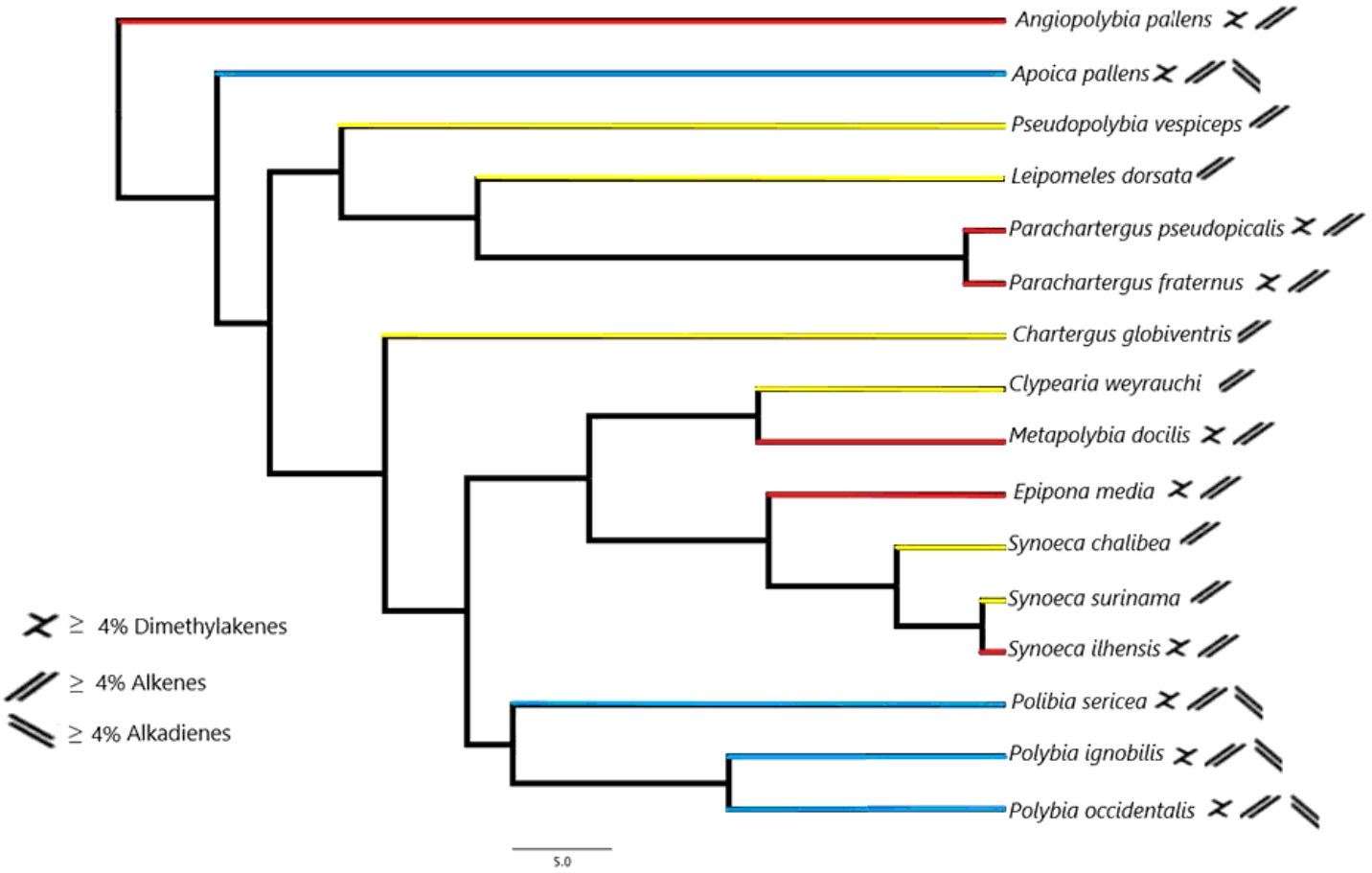

Figure 2

Phylogenetic tree according to Menezes et al. (2020) and the class relationships of CHCs among species of the Epiponini tribe. Colors represent three classes of phylogenetically informative CHs distributed among the species of the Epiponini tribe according to Menzel et al. (2017). Red: Genera that have only alkenes and dimethylalkanes; Blue: Genera that have the three classes in their profile, and; Yellow: Genera that have only alkenes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableII.docx
- TableSuplementary.xlsx