

## Research Paper

**Cite this article:** Santos GKN, Navarro DMdoAF, Maia ACD (2024). Cuticular lipid profiles of selected species of cyclocephaline beetles (Melolonthidae, Cyclocephalini). *Bulletin of Entomological Research* **114**, 124–133. <https://doi.org/10.1017/S0007485323000664>

Received: 7 July 2023  
Revised: 19 September 2023  
Accepted: 30 November 2023  
First published online: 25 January 2024


### Keywords:

alkenes; chemotaxonomy; contact pheromones; florivory; pollination; scarabaeidae

### Corresponding author:

Artur Campos D. Maia;  
Email: [arturmaia@gmail.com](mailto:arturmaia@gmail.com)

# Cuticular lipid profiles of selected species of cyclocephaline beetles (Melolonthidae, Cyclocephalini)

Geanne Karla N. Santos<sup>1,2</sup>, Daniela Maria do Amaral F. Navarro<sup>2</sup> and Artur Campos D. Maia<sup>3,4</sup> 

<sup>1</sup>Secretaria Executiva de Meio Ambiente de Paulista (SEMA), Prefeitura Municipal do Paulista, Paulista, 53401-441, Brazil; <sup>2</sup>Department of Fundamental Chemistry, Centro de Ciências Exatas e da Natureza, Universidade Federal de Pernambuco, Recife, 50740-560, Brazil; <sup>3</sup>Department of Zoology, Centro de Biociências, Universidade Federal de Pernambuco, Recife PE, 50670-901, Brazil and <sup>4</sup>Laboratory of Sciences for the Environment, University of Corsica, UMR 6134 SPE, Ajaccio, France

## Abstract

Neotropical cyclocephaline beetles, a diverse group of flower-loving insects, significantly impact natural and agricultural ecosystems. In particular, the genus *Cyclocephala*, with over 350 species, displays polymorphism and cryptic complexes. Lacking a comprehensive DNA barcoding framework, accessible tools for species differentiation are needed for research in taxonomy, ecology, and crop management. Moreover, cuticular hydrocarbons are believed to be involved in sexual recognition mechanisms in these beetles. In the present study we examined the cuticular chemical profiles of six species from the genus *Cyclocephala* and two populations of *Erioscelis emarginata* and assessed their efficiency in population, species, and sex differentiation. Overall we identified 74 compounds in cuticular extracts of the selected taxa. Linear alkanes and unsaturated hydrocarbons were prominent, with ten compounds between them explaining 85.6% of species dissimilarity. Although the cuticular chemical profiles efficiently differentiated all investigated taxa, only *C. ohausiana* showed significant cuticular profile differences between sexes. Our analysis also revealed two *E. emarginata* clades within a larger group of ‘*Cyclocephala*’ species, but they were not aligned with the two studied populations. Our research underscores the significance of cuticular lipid profiles in distinguishing selected cyclocephaline beetle species and contemplates their potential impact as contact pheromones on sexual segregation and speciation.

## Introduction

The evolutionary success of insects in terrestrial environments is indisputably related to their ability to conserve water (Garwood and Edgecombe, 2011). One of the factors that contribute to this physiological trait is the presence of a cuticle that protects them against desiccation. The insect cuticle is a structure composed of several layers, among which the most efficient against water loss is the lipid layer associated with the epicuticle (Hadley, 1980). It might contain saturated or unsaturated hydrocarbons, branched or not, free fatty acids, aldehydes, alcohols, esters, ketones, glycerides, and sterols (Blomquist, 2010).

Among the cuticular lipids, hydrocarbons deserve special attention because they are often present in high concentrations and play important roles in intra- and interspecific communication in insects (Lockey, 1991; Singer, 1998; Howard and Blomquist, 2005). Cuticular hydrocarbons (CHCs) in insects are present as a complex mixture of molecules, often characteristic of each species (Bagnères and Wicker-Thomas, 2010). Within the same species, the CHC composition may vary between sexes (Steiger and Stöckl, 2014) and in relation to different life cycle stages (Lockey, 1991; Howard and Blomquist, 2005; Thomas and Simmons, 2008). Other factors such as the insects’ diet (Liang and Silverman, 2000; Ingleby, 2015), reproductive stage (Scott *et al.*, 2008; Caselli *et al.*, 2023), different populations of the same species (Hirai *et al.*, 2008), and different colonies of social insects (Jutsum *et al.*, 1979; Morel *et al.*, 1988; Dahbi *et al.*, 1996) as well as different castes in the same colony (Wagner *et al.*, 1998; Greene and Gordon, 2003) can alter the CHC composition both quanti- and qualitatively.

Regarding their structure, CHCs have carbon chains ranging from 11 to 43 atoms, both even and odd numbered. They are distributed into three main classes: n-alkanes, olefins, and methyl-branched alkanes. Olefins and methyl-branched alkanes usually occur as isomeric mixtures (Lockey, 1991; Blomquist, 2010). The most common olefins in insects are alkenes, but compounds with two or, less frequently, more unsaturations may also be present. The position of the double-bonds in the carbon chain is of key relevance to their biological activities

and is usually experimentally determined through derivatisation of the unsaturated compounds, followed by analysis by GC-MS (Lockey, 1988; Blomquist, 2010).

Due to their taxon-specific character, the chemical profiles of insects' cuticles can be used to assist in species identification along with other more conventional taxonomic approaches (i.e., morphological characters and molecular biology) (Kather and Martin, 2012). In fact, their efficiency as a chemotaxonomic tool in insect research has been demonstrated in identifying cryptic species (Soon *et al.*, 2021) and initial trends in reproductive isolation among populations (Hartke *et al.*, 2019).

Cyclocephaline beetles (Melolonthidae, Cyclocephalini) comprise a diverse group of essentially Neotropical Scarabaeoidea, currently encompassing ~500 spp. (Moore *et al.*, 2018a). Some have rhizophagous larvae, commonly referred to as white grubs, which are widely known as pests in crops, lawns, and reforestation areas (Moore *et al.*, 2018b). The adults of nine of the 14 currently acknowledged genera within the tribe are highly specialised pollinators and florivores that are attracted to their hosts by floral fragrances (Maia *et al.*, 2012, 2018; Moore *et al.*, 2018b). The functional details behind specific sexual recognition in these insects have only been superficially assessed (Nóbrega *et al.*, 2022), but it is generally agreed that close-range signals (i.e., visual, tactile, and/or chemical) might be involved.

The taxonomy of cyclocephaline beetles at the species level is primarily dependent on male genitalia characters (Endrödi, 1985; Moore *et al.*, 2018b). Therefore, it requires precise dissection, assembly, and analysis of anatomical structures, making classification challenging or even unfeasible if only females are available. Various species of the most speciose genus in the tribe, *Cyclocephala* (> 350 spp.), display multiple levels of polymorphism (Ratcliffe *et al.*, 2013, 2020), whereas others form morphologically indistinguishable complexes with cryptic species (Neita-Moreno, 2021). While an integrative DNA barcoding framework for the tribe Cyclocephalini has not yet been examined (Moore *et al.*, 2018b; Ratcliffe *et al.*, 2020), alternative accessible tools for species differentiation could advance research not only in taxonomy but also in ecology and crop management (Lyal *et al.*, 2008; Lagomarsino and Frost, 2020).

In the present study, we investigated the cuticular chemical profiles of six species within the genus *Cyclocephala* and two distinct populations of one species within the genus *Erioscelis*. We aimed to address the following questions: (i) Is it possible to discriminate between species based on their cuticular chemical profiles?; (ii) Are there any sex-related differences in these profiles?

## Materials and methods

### Investigated species and collection sites

*Cyclocephala celata* Dechambre, 1980: This species is found from the northeastern Atlantic Coast of Brazil across dry seasonal forests of South America to Bolivia and Paraguay (Endrödi, 1985; Moore *et al.*, 2018a). Adults are medium-sized, measuring about 15–17 mm in length. Known pollinators of Annonaceae and Araceae (Maia *et al.*, 2012; Parizotto and Grossi, 2019). Specimens used in the present study were collected in inflorescences of *Philodendron acutatum* Schott (Araceae) between March and June 2014 in the municipality of Igarassu, state of Pernambuco (7°49'S, 35°02'W; ca. 110 m.a.s.l.) (refer to Maia *et al.*, 2010 for details).

*Cyclocephala cearae* Höhne, 1923: This species is endemic to northeastern Brazil where it is found from the coastal Atlantic Forest to the Caatinga (Endrödi, 1985; Moore *et al.*, 2018a). Adults are medium-sized, measuring about 17–18 mm in length. Known pollinators of Araceae (Maia *et al.*, 2013). Specimens for the present study were collected in inflorescences of *Taccarum ulei* Eng. & K. Krause between March and June 2014 in the municipality of Igarassu, state of Pernambuco (7°47'S, 34°55'W; ca. 80 m.a.s.l.) (refer to Maia *et al.*, 2013 for details).

*Cyclocephala paraguayensis* Arrow, 1903: This species is widely distributed in South America, found north from the Colombian Orinoquia to the Chacoan Argentina and Uruguay in the south (Endrödi, 1985; Moore *et al.*, 2018a). Adults are relatively small, measuring about 11–13 mm in length. Specialised florivores associated with cacti and cultivated plants (Moore and Jameson, 2013). Specimens used in the present study were collected with light traps installed in a sugarcane field between March and June 2014 in the municipality of Igarassu, state of Pernambuco (7°49'S, 35°02'W; ca. 110 m.a.s.l.) (refer to Maia *et al.*, 2010 for details).

*Cyclocephala distincta* Burmeister, 1847: This species is widely distributed in South America, found north from Colombia and the Guianas to the southern Brazilian state of Santa Catarina (Endrödi, 1985; Moore *et al.*, 2018a). Adults are small, measuring about 9–10 mm in length. Specialised florivores associated with palms (Moore and Jameson, 2013; Maia *et al.*, 2018). Specimens used in the present study were collected with scent-baited traps (e.g., 2-isopropyl-3-methoxypyrazine see Maia *et al.*, 2018 for details) installed in a sugarcane field between December 2016 and March–April 2017 in the municipality of Igarassu, state of Pernambuco (7°49'S, 35°02'W; ca. 110 m.a.s.l.) (refer to Maia *et al.*, 2010 for details).

*Cyclocephala forsteri* Endrödi, 1963: This species is widely distributed in South America, found north from the Colombian Orinoquia to the southern Brazilian state of Santa Catarina (Moore *et al.*, 2018a). Immatures are rhizophages associated with soy crops (Santos and Ávila, 2007). Adults are large-sized beetles, measuring about 20–22 mm in length. Specialised florivores associated with palms (Oliveira and Ávila, 2011; Maia *et al.*, 2020). Specimens used in the present study were collected with scent-baited traps (e.g., 4-methylanisole + 2-isopropyl-3-methoxypyrazine + 2-*sec*-butyl-3-methoxypyrazine) installed in a rural area ca. 30 km southeast from the municipality of Campo Grande, state of Mato Grosso do Sul (20°37'S 54°31'W; ca. 590 m.a.s.l.), between October and November 2015 (refer to Maia *et al.*, 2020 for details).

*Cyclocephala ohausiana* Höhne, 1923: This species is native to the Cerrado savanna in central Brazil, as well as its transitional zone with the Atlantic Forest (Endrödi, 1985; Moore *et al.*, 2018a). Adults are medium-sized beetles, measuring about 15–17 mm in length. Pollinators of Annonaceae (Moore and Jameson, 2013; Costa *et al.*, 2017). Specimens used in the present study were collected in flowers of *Annona coriacea* Mart. (Annonaceae) between October and November 2015 in a rural area ca. 30 km southeast from the municipality of Campo Grande, state of Mato Grosso do Sul (20°37'S 54°31'W; ca. 590 m.a.s.l.) (refer to Maia *et al.*, 2020 for details).

*Erioscelis emarginata* (Mannerheim, 1829): This species is found from the coastal Atlantic Forest west to the Cerrado in Brazil, from the Chocoan Argentina to Ecuador. Adults are large-sized beetles, measuring about 21–24 mm in length. Known pollinators of Araceae (Moore and Jameson, 2013). Specimens used

in the present study were collected in inflorescences of *Philodendron bipinnatifidum* Schott ex Endl. and *P. mello-barretoanum* R. Burle-Marx ex G.M. Barroso at two distinct locations in central Brazil. The first in the municipality of Taguatinga, Federal District, in December 2013 (15°51'S, 48°01'W; ca. 1120 m.a.s.l.). The second in the municipality of Nova Odessa, state of São Paulo, in October 2014 (22°46'S, 47°18'W; ca. 580 m.a.s.l.) (refer to Barros *et al.*, 2020 for details). We considered the two collection sites as separate populations of *E. emarginata*, hereby referred to as 'Taguatinga' and 'Nova Odessa'.

Upon collection the beetles were placed in semi-transparent plastic containers (30 × 21 × 21 cm) with perforated lids and filled with sieved and moistened pot soil. The containers were maintained at a temperature of 27 ± 2°C, relative humidity of 70 ± 5%, and a photoperiod of 14 hours of light and 10 hours of darkness for 24 to 96 hours before processing (as described below). The beetles were provided with fresh apple slices for food, which were available *ad libitum*.

Species identifications were conducted by Professor Paschoal Coelho Grossi, curator of the Entomological Collection at the Federal Rural University of Pernambuco (CERPE) and an expert in Dynastinae taxonomy. Voucher specimens for each species have also been deposited at CERPE.

### Sampling and analysis of cuticular extracts

Female and male specimens of the seven investigated species, including two distinct populations of *E. emarginata*, were placed either individually or in batches of 10 (for *C. distincta* and *C. paraguayensis*) into clean 5 mL borosilicate glass vials (Sigma-Aldrich, USA). Rapid gradual freezing (−5 to −20°C; 60 min) was used to euthanise the beetles, which were then rinsed with deionised H<sub>2</sub>O to remove soil and food particles adhered to the cuticle surface. Subsequently, they were placed on clean absorbent paper to remove excess water, and dried using a stream of N<sub>2</sub>. After drying, the specimens were put in clean borosilicate glass 5 mL vials, containing 1 ml (individual) or 2.5 ml (batch) of n-hexane (HPLC grade, bidistilled) and swirled gently for one minute. Preliminary results showed that a short-lasting extraction time minimises the interference from non-cuticular lipids, eliminating the need for a chromatographic column to purify the eluates.

The crude eluates were finally filtered using a borosilicate glass wool plug (Sigma-Aldrich, USA) packed inside a borosilicate glass Pasteur pipette into 2.0 ml (individual) or 4.0 ml (batch) chromatography vials. The extracted eluates from the batches of 10 individuals were additionally concentrated under a laminar N<sub>2</sub> flow to a final volume of 0.5 ml. All samples were stored under −24°C for 5 to 160 days until further processing.

The samples were analysed by gas chromatography coupled to mass spectrometry (GC-MS) using an Agilent 5975C Series GC/MSD quadrupole system (Agilent Technologies, Palo Alto, USA) equipped with an HP-5 ms non-polar column (Agilent J&W; 30 m × 0.25 mm i.d., 0.25 µm film thickness). An aliquot of 1.0 µl of each sample was injected in splitless mode. The GC oven temperature was set at 60°C for 1 min, then increased at a rate of 15°C min<sup>−1</sup> up to 260°C, and kept for 20 min. The carrier gas (He) flow was kept constant at 1 ml min<sup>−1</sup>. MS source and quadrupole temperatures were set respectively at 230 and 150°C, and the mass spectra were recorded at 70 eV (EI mode) with a scanning speed of 0.5 scan/s from *m/z* 35 to 550. The compounds were identified by comparing their mass spectra and retention times to those of authentic standards available in the

MassFinder 4, NIST20, Wiley Registry™ 9th Edition, and MACE\_R4 (Schulz and Möllerke, 2022) reference libraries, incorporated in the software Agilent MSD Productivity ChemStation. Identification of double-bond positions in 7- and 9-alkenes was achieved through sample derivatisation (Francis and Veland, 1987). The peak areas in the chromatograms were integrated to obtain the total ionic signal, and their values were used to determine the relative proportions of each compound. A homologous series of linear alkanes (C<sub>9</sub> – C<sub>40</sub>) was used to determine linear retention indices (RIs) (van Den Dool and Kratz, 1963) and provide a means for comparison with data available in the literature. Unassigned compounds corresponding to < 1% of the total individual peak area in at least one of the analysed samples were pooled.

We used the main compounds for comparative statistical analyses (see below), selected through an arbitrary threshold of ≥ 2% relative concentration (total individual peak area) in at least one of the analysed samples.

### Statistical analyses

To assess differences in the cuticular lipid profiles of the seven investigated species, we first subjected the compositional chemical data (relative proportion of compounds in the samples) to a centred log ratio (clr) transformation with a 0.001 threshold to minimise misinterpretations caused by a constrained data set (as reviewed by Brückner and Heethoff, 2017). We then used the clr-transformed data to generate a two-way paired group (UPGMA) hierarchical clustering and heatmap using the Euclidean similarity index. This approach allowed us to track the influence of individual chemical compounds on the species' dendrogram.

We conducted a global permutational multivariate analyses of variance (PERMANOVA; Pseudo-*F* value) for the discrimination of groups/clusters recovered in the two-way UPGMA and a permutational analysis of multivariate dispersions (PERMDISP; *F* value) to elucidate the dispersion of the PERMANOVA.

Additionally, we ran pairwise PERMANOVA tests with the raw compositional chemical data (Bray-Curtis dissimilarity index) to assess differences between sexes ('female' vs 'male', except for *C. paraguayensis*), and populations ('Taguatinga' vs 'Nova Odessa', for *E. emarginata* only). We also conducted similarity percentages tests (SIMPER) with the raw compositional chemical data to determine the contribution of individual compounds to differences in chemical profiles among species, as well as between genera, sexes, and populations, emphasising quantitative trends. All statistical analyses were performed using PAST v.4.11 (Hammer *et al.*, 2001). Figures and graphs were edited using Adobe Illustrator 2023 (Adobe Systems Inc.).

### Results

We identified 74 compounds in the solvent cuticular extracts of adult *Cyclocephala* spp. (*n* = 50 samples, six spp.) and *E. emarginata* (*n* = 51 samples, two populations). Linear alkanes (C<sub>21</sub> to C<sub>34</sub>), particularly odd-chain C<sub>21</sub> – C<sub>29</sub>, and unsaturated hydrocarbons, especially (*Z*)-7 and (*Z*)-9 odd-chain C<sub>21</sub> – C<sub>35</sub> alkenes, were the most well-represented cuticular lipids in all analysed samples, ranging from 94.7 to 33.2% and 66.2 to 5.1% relative composition, respectively. Fatty acyls (mostly acids and esters) and methyl-branched alkanes corresponded from null to 12.0 and 10.2%, respectively (table 1).

**Table 1.** Average relative amounts (%) of cuticular lipids ordered per chemical class in hexane extracts of female (♀) and male (♂) adult specimens of *Cyclocephala* spp. and *Erioscelis emarginata* (Melolonthidae, Dynastinae, Cyclocephalini).

Genus/species		<i>Cyclocephala</i>										<i>Erioscelis emarginata</i>					
		Species/population		<i>C. cearae</i>		<i>C. celata</i>		<i>C. distincta</i>		<i>C. forsteri</i>		<i>C. ohausiana</i>		<i>C. paraguayensis</i>		Taguatinga pop.	
RI	Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
	Number of samples	5	5	5	3	5	3	5	5	4	8	2		8	12	17	14
Alkanes																	
1500	Pentadecane	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
1600	Hexadecane	-	-	-	-	-	-	0.1	0.1	0.1	0.1	-	-	-	-	-	-
1700	Heptadecane	-	-	-	-	-	-	0.2	0.1	0.3	0.5	-	-	-	-	-	-
1800	Octadecane	-	-	-	-	-	-	0.2	0.1	0.1	0.2	-	-	-	-	-	-
1900	Nonadecane	-	-	-	-	-	-		0.2	0.4	0.1	-	-	-	-	-	-
2000	Eicosane	-	-	-	-	-	-			0.2	0.1	-	-	-	-	-	-
2100	<b>Heneicosane*</b>	0.2	0.2	5.2	6.7	-	-	0.3	0.2	30.8	19.5	-	-	-	-	-	-
2200	Docosane	0.1	0.1	0.4	0.5	-	-	0.2	0.1	0.2	0.3	0.1	-	-	-	-	-
2300	<b>Tricosane*</b>	13.7	16.1	19.8	23.7	8.6	7.8	20.8	10.4	5.3	19.9	4.1	5.8	9.6	2.4	2.5	
2400	Tetracosane	0.4	0.4	-	-	0.4	0.3	0.8	0.6	-	-	0.4	0.3	0.6	-	0.1	
2500	<b>Pentacosane*</b>	11.3	12.8	6.4	9.2	19.2	21.3	16.6	15.5	-	-	33.2	19.6	30.6	11.2	15.1	
2600	Hexacosane	0.1	-	-	-	0.2	0.2	0.4	0.3	-	-	0.3	0.9	0.8	0.7	0.8	
2700	<b>Heptacosane*</b>	6.8	6.7	4.4	4.8	14.9	17.8	8.1	10.6	-	-	21.6	52.0	36.5	74.5	72.1	
2800	Octacosane	-	-	-	-	-	-	0.1	0.2	0.2	0.3	0.2	0.2	0.1	0.2	0.4	
2900	<b>Nonacosane</b>	-	-	3.6	6.7	5.6	7.5	2.6	6.1	-	-	7.9	3.5	2.9	4.7	3.7	
3100	Hentriacontane	0.6	-	-	0.9	0.3	0.5	0.4	1.1	-	-	-	-	-	-	-	
Unsaturated hydrocarbons																	
1883	(Z)-7-Nonadecene	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	
1976	(Z)-9-Eicosene	-	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	
2072	<b>Heneicosadiene</b>	-	-	-	-	-	-	-	-	11.8	0.3	-	-	-	-	-	
2077	<b>(Z)-9-Heneicosene*</b>	-	-	-	-	-	-	-	-	43.1	2.0	-	-	-	-	-	
2083	(Z)-7-Heneicosene	-	-	-	-	-	-	-	-	2.3	1.7	-	-	-	-	-	
2177	(Z)-9-Docosene	-	-	0.5	0.4	-	-	-	-	0.2	0.2	-	-	-	-	-	
2183	(Z)-7-Docosene	-	-	-	-	-	-	-	-		0.1	-	-	-	-	-	
2274	Tricosadiene	-	-	-	-	-	-	-	-	0.7	10.2	-	-	-	-	-	

(Continued)

Table 1. (Continued.)

Genus/species	<i>Cyclocephala</i>												<i>Erioscelis emarginata</i>			
	Species/population		<i>C. cearae</i>		<i>C. celata</i>		<i>C. distincta</i>		<i>C. forsteri</i>		<i>C. ohausiana</i>		<i>C. paraguayensis</i>		Taguatinga pop.	São Paulo pop.
2277	<b>(Z)-9-Tricosene*</b>	1.6	1.6	44.1	31.1	-	-	0.7	0.9	1.8	24.8	-	-	-	-	-
2283	<b>(Z)-7-Tricosene</b>	-	-	-	-	-	-	0.1	-	0.9	12.4	-	-	-	-	-
2375	(Z)-9-Tetracosene	0.3	0.3	-	-	-	-	0.3	0.3	-	-	-	-	-	-	-
2385	(Z)-7-Tetracosene	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2477	<b>(Z)-9-Pentacosene*</b>	29.1	31.8	5.5	3.2	3.5	1.9	34.3	33.5	-	-	-	-	-	-	-
2484	<b>(Z)-7-Pentacosene*</b>	13.2	8.9	-	-	6.6	6.3	-	-	-	-	-	-	-	-	-
2532	Hexacosadiene	-	-	-	-	-	-	-	-	-	1.0	-	-	-	-	-
2533	Hexacosene	-	-	-	-	-	-	0.3	-	-	1.2	-	-	-	-	-
2544	Hexacosene	-	-	-	-	-	-	-	-	-	0.9	-	-	-	-	-
2676	<b>(Z)-9-Heptacosene</b>	6.0	6.2	3.0	1.7	4.9	3.4	3.3	3.1	-	-	-	1.0	3.0	0.2	0.1
2683	(Z)-7-Heptacosene	4.3	3.8	-	-	2.6	2.6	0.1	-	-	-	-	0.1	0.2	0.1	-
2867	Nonacosene	-	-	-	-	-	-	0.2	0.4	-	-	-	-	-	-	-
2876	<b>(Z)-9-Nonacosene*</b>	0.5	0.4	4.8	2.7	18.2	15.4	1.6	2.1	-	-	-	5.3	5.1	1.9	0.7
2885	<b>(Z)-7-Nonacosene</b>	5.2	4.3	-	-	-	-	0.1	0.1	-	-	-	0.1	0.1	0.2	-
3028	Hentriacontadiene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3052	Hentriacontadiene	-	-	-	-	0.3	0.2	-	-	-	-	0.7	-	-	-	-
3064	Hentriacontene	-	-	-	-	-	-	0.0	-	-	-	0.2	-	-	-	-
3074	<b>(Z)-9-Hentriacontene*</b>	5.8	5.3	0.7	3.6	11.6	12.5	0.7	1.9	-	-	0.7	11.2	10.3	3.9	4.3
3082	(Z)-7-Hentriacontene	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-
3251	<b>Tritriacontadiene</b>	-	-	-	-	-	-	-	-	-	-	7.4	-	-	-	-
3258	Tritriacontadiene	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-
3270	(Z)-9-Tritriacontene	-	-	-	-	-	-	-	-	-	-	3.4	-	-	-	-
3281	(Z)-7-Tritriacontene	-	-	-	-	-	-	-	-	-	-	1.1	-	-	-	-
3449	Pentatriacontadiene	-	-	-	-	-	-	-	-	-	-	2.3	-	-	-	-
3468	(Z)-9-Pentatriacontene	-	-	-	-	-	-	-	-	-	-	3.2	-	-	-	-
Fatty acyls																
1795	Ethyl tetradecanoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1825	Hexadecenal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1890	Ethyl pentadecanoate	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-
1926	Methyl hexadecanoate	-	-	-	-	-	-	0.2	0.3	-	-	-	-	-	-	-

1959	Hexadecanoic acid*	-	-	-	-	-	-	-	-	-	3.5	-	-	-	-	
1977	Ethyl (Z)-hexadec-9-enoate	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	
1991	Ethyl hexadecanoate	-	-	-	-	0.1	0.1	0.1	0.5	-	-	0.8	-	-	-	-
2025	Isopropyl hexadecanoate	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-
2105	Methyl (Z)-octadec-9-enoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2130	Methyl octadecanoate	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
2140	(Z)-Octadec-9-enoic acid*	-	-	-	-	-	-	0.1	-	-	-	5.1	-	-	-	-
2160	Octadecanoic acid	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-
2173	Ethyl (Z)-octadec-9-enoate	-	-	-	-	0.5	0.9	-	-	-	-	2.1	-	-	-	-
2189	Butyl hexadecanoate	0.1	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-
2195	Ethyl octadecanoate	-	-	-	-	-	0.1	-	0.1	-	-	0.1	-	-	-	-
2223	Propan-2-yl octadecanoate	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-
Methylbranched alkanes																
1706	Pristane	-	-	-	-	-	-	0.3	0.2	0.7	1.1	-	-	-	-	-
1720	<i>m/z</i> : 97,57,99,43,41	-	-	-	-	-	-	0.1	-	-	2.2	-	-	-	-	-
1745	Farnesane	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-
1810	Phytane	-	-	-	-	-	-	0.4	0.2	0.2	0.5	-	-	-	-	-
2728	<b><i>m/z</i>: 57,71,85,43,55</b>	-	-	-	-	1.4	0.7	5.3	8.0	-	-	-	-	-	-	-
2826	Squalene	-	-	-	-	-	-	-	1.1	-	-	-	-	-	-	0.1
2925	Methylnonacosane	-	-	-	-	0.4	0.1	0.8	0.7	-	-	-	-	-	-	-
<b>Other compounds</b>		0.5	0.5	1.6	4.8	0.3	0.3	0.4	0.4	0.1	0.3	-	-	0.2	-	0.1

Individuals from two populations were analysed for *E. emarginata* (see Materials and methods for details). Linear retention indices (RIs) were calculated based on a homologous series of linear alkanes (C9 – C40) (van Den Dool and Kratz, 1963) and provide a means for comparison with data available in the literature. The identities of (Z)-7 and (Z)-9-alkenes were confirmed by DMDS derivatisation and comparison with RI data in mass spectral libraries (see Materials and methods for details). Compounds in bold lettering were identified in > 5% average relative concentration in at least one species and/or population. The 10 compounds marked with an asterisk (\*) are the main contributors to overall interspecific dissimilarity (see Results for details). Only compounds accounting for at least 1% average amount in any species are presented. Unassigned and identified compounds below this threshold were pooled as 'Other compounds' and their total relative percentages added. Also refer to Table Data S1 for more details.

The cuticular chemical profiles significantly differed among the seven investigated species, as evidenced by PERMANOVA (Pseudo- $F=70.04$ ,  $P<0.0001$ ; pairwise Pseudo- $F\geq 13.83$ ,  $P<0.01$ ) and PERMDISP ( $F=67.12$ ,  $P<0.0001$ ; pairwise  $F\geq 18.31$ ,  $P<0.01$ ). Overall, the qualitative and quantitative differences in the relative concentrations of ten compounds alone (four alkanes, six (Z)-alkenes) explained 85.6% of the dissimilarity among species.

The only species in which the cuticular chemical profiles significantly differed between sexes was *C. ohausiana* (Pseudo- $F=64.52$ ,  $P<0.0001$ ), with an overall average dissimilarity of 65.2% that was mostly explained by differential occurrence and relative proportions of  $C_{21}$  and  $C_{23}$  (Z)-alkenes and linear alkanes, as well as a heneicosadiene of unknown structure (RI 2072) (refer to table 1 for details).

In the UPGMA dendrogram and heatmap, we can visually observe a distinct separation of all examined taxa into discrete clades (fig. 1). Notably, we identified two sister clades of *E. emarginata* within a larger group that encompasses all *Cyclocephala* species. It is worth highlighting that these two *E. emarginata* clades did not correspond to the 'Nova Odessa' and 'Taguatinga' populations we investigated, as the largest clade included samples from both populations (fig. 1). Nevertheless, a pairwise PERMANOVA test provided statistical support for the differentiation between these two populations (Pseudo- $F=61.14$ ,  $P<0.0001$ ).

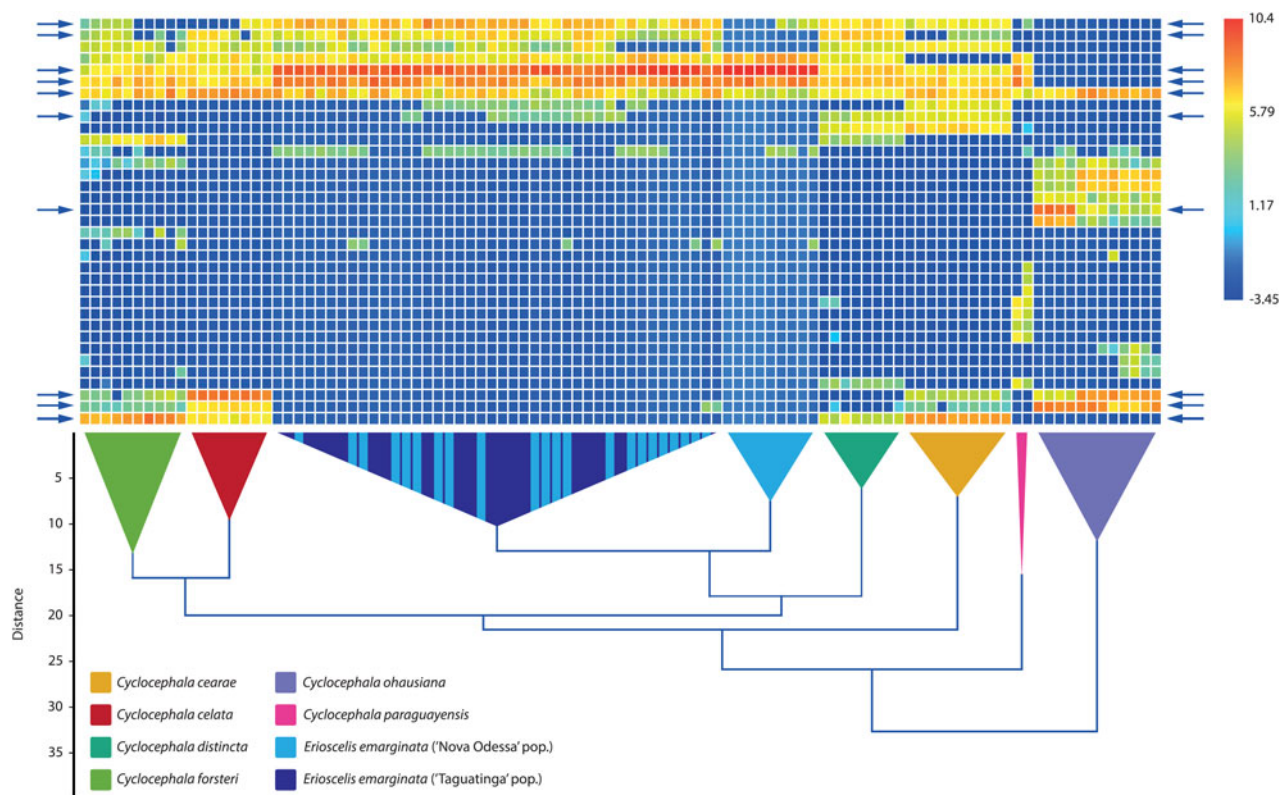
## Discussion

The cuticular lipid profiles of the seven *Cyclocephalini* species investigated in our study exhibited high relative concentrations

of odd, very long-chain ( $>C_{20}$ ; VLC) n-alkanes and (Z)-alkenes. These compounds are widely found in the epicuticles of various insect groups, including Scarabaeoid beetles (Blomquist, 2010). Interestingly, the identities and relative proportions of these compounds were highly effective in explaining the well-supported species groupings observed in our study. In fact, a mere ten compounds (out of 77 identified in total) accounted for over 85% of the dissimilarity among species.

In a chemotaxonomic analysis of Mediterranean earth-boring dung beetles (Geotrupidae), Niogret *et al.* (2019) identified the presence of n-alkanes and (Z)-alkenes in the cuticular lipid profiles of all 12 species studied. The relative concentrations of these compounds varied, ranging from 4.8 to 17.3% for n-alkanes and 0.9 to 14.0% for (Z)-alkenes. Similar findings were reported by Fletcher *et al.* (2008) in a study involving six Australian species of Scarabaeidae, where all species except one exhibited the presence of n-alkanes or (Z)-alkenes in their cuticular profiles. However, unlike our study, both investigations found that methyl-branched alkanes were also significantly abundant, accounting for anywhere between 12 and 83% of the relative concentration of cuticular lipids.

Although VLC-alkanes are commonly found in the epicuticles of insects (Blomquist, 2010) and have been identified as pheromonal components in various taxa (El-Sayed, 2023), their precise role in chemical communication among beetles remains poorly understood (Howard and Blomquist, 2005). It is widely recognised that the molecular discriminative features of VLC-alkanes are relatively limited, being primarily based on carbon-chain length (van Zweden and d'Ettorre, 2010). The variation in



**Figure 1.** Paired group (UPGMA) two-way dendrogram and heat map of the cuticular chemical profiles of *Cyclocephala* spp. and *Erioscelis emarginata* (Melolonthidae, Dynastinae, Cyclocephalini) using centred log ratio (clr)-transformed compositional chemical data (relative proportion of compounds in the samples). Each column correspond to one sample (see Materials and methods for details) and each row to a cuticular lipid identified by GC-MS analysis. Arrows mark the rows of the compounds that contributed most to clade formation in a similarity percentages test (SIMPER) with the raw compositional chemical data. Only compounds accounting for at least 2% average amount in any species (35 in total) were used for the analysis (see Materials and methods for details).

VLC-alkane proportions observed across different insect species, populations, sexes, and castes, nonetheless, provides support for their influence on behavioural modulation (van Zweden and d'Ettoire, 2010).

On the other hand, (Z)-alkenes and methyl-branched alkanes possess greater potential as informative semiochemicals based on their molecular structure. This is attributed to factors such as the position of the methyl group or double-bond, and stereoisomerism (van Zweden and d'Ettoire, 2010). In the case of beetles, (Z)-7 and (Z)-9-alkenes with odd-chain lengths ranging from C<sub>23</sub> to C<sub>29</sub> have been found to elicit contact-based sexually-oriented behaviour in various species of Cerambycidae (as reviewed by Ginzl, 2010). Therefore, it is plausible that the presence of these compounds in the cuticular lipid profiles of Scarabaeoidea species could serve similar functions. The same can be said for methyl-branched alkanes, which can act as highly specific constituents of sex or aggregation pheromones due to their variability and chiral nature (van Zweden and d'Ettoire, 2010). While our current findings are derived from a limited sampling effort, we propose further exploration of the potential involvement of unsaturated and methyl-branched alkanes in contact-based chemical communication among cyclocephaline beetles. In this context, it is also worth highlighting the alkadienes present in significant relative proportions in the cuticular lipid profiles of two of the studied species, namely *C. ohausiana* and *C. paraguayensis*.

We were surprised to find that sex-related differences were only evident in one of the taxa investigated in our study. This finding is intriguing considering the highly aggregative behaviour observed in flower-loving species of cyclocephaline beetles, often comprised by hundreds (Gottsberger and Silberbauer-Gottsberger, 1991; Scariot *et al.*, 1991), even thousands (Henderson, 1986; Núñez, 2014), of individuals. It is also worth noting that these aggregations regularly consist of multiple species coexisting (Beach, 1982; Young, 1986; Maia *et al.*, 2013; Costa *et al.*, 2017), a context that has led to speculation about the potential role of contact or short-range chemical communication in sex recognition among these beetles (Nóbrega *et al.*, 2022). Such communication mechanisms could serve to prevent both same-sex pairing and interspecific copulation.

Indirect evidence of intraspecific contact chemical communication in cyclocephaline beetles has been obtained through controlled rearing experiments involving various species of *Cyclocephala* (Rodrigues *et al.*, 2010; Nogueira *et al.*, 2013; Souza *et al.*, 2014). These experiments have demonstrated successful copulation and egg fertilisation occurring in small aggregations or even in one-on-one pairings, indicating at least some level of sex differentiation. Notably, Nóbrega *et al.* (2022) propose that male *C. distincta* are capable of distinguishing conspecific females upon contact, and that specific innate female traits trigger male sexual behaviour. This suggests that contact-based chemical cues play a crucial role in facilitating reproductive interactions within the species.

It is important to note that when examining trap collections and directly assessing insects within flowers, a consistent observation is that the female-to-male sex ratios of cyclocephaline beetles typically range from 1:1 to 2:1 (Beach, 1982; Albuquerque *et al.*, 2016; Maia *et al.*, 2020). This finding indicates that, on average, males within species-specific aggregations achieve a copulation success rate of approximately 50 to 75% from their random mating attempts.

It is likely that within the range of interactions between cyclocephaline beetles and their host plants, spanning from highly

specialised (monophily) to more generalised (polyphily), species with a narrower preference (oligophilous) are increasingly dependent on floral scents to ensure their reproductive success. This scenario seems to be evident in diverse coastal forest ecosystems of French Guiana, where several co-flowering species of Araceae (such as *Philodendron*, *Dieffenbachia*, and *Montrichardia*) are each exclusively visited by a unique species or subset of species (Gibernau *et al.*, 1999, 2003; Gibernau and Barabé, 2002; Gibernau, 2015). This intricate interplay between specific floral hosts and distinct beetle species underscores the importance of chemical signals in facilitating successful reproductive interactions.

A contrasting scenario unfolds in the interactions involving large-flowered *Annona* spp., specifically *A. coriacea*, in the Cerrado savanna ecosystems of central Brazil. These nocturnally blooming flowers attract at least seven potentially syntopic species of *Cyclocephala*, including *C. ohausiana* (Costa *et al.*, 2017). Within the floral chambers of *Annona* spp., multiple congeneric species often aggregate together, creating a complex ecological milieu. This coexistence of closely related species may impose selective pressures that drive the development of more efficient mechanisms for contact-based sex discrimination. It is conceivable that these selective pressures contribute to the observed significant sex differences in the cuticular lipid profiles of *C. ohausiana*.

Our research findings underscore the significance of cuticular lipid profiles as robust tools for distinguishing among specific flower-loving cyclocephaline beetle species. These findings hold particular relevance in the identification of cryptic species groups, notably within the *Cyclocephala* genus, such as the black species documented by Neita-Moreno (2021), as well as the 'sexpunctata' and 'latericia' species complexes (Moore, 2011; Santos, 2014), among others. Moreover, our study emphasises the imperative need for further research to establish appropriate comparative methodologies, enabling the investigation of variations in contact communication among cyclocephaline beetle species in diverse ecological conditions. Such research is pivotal in comprehending the spectrum of interactions involving cyclocephaline beetles and their floral hosts, ranging from monophily to polyphily. The chemotaxonomic characteristics of lipid profiles in cyclocephaline beetles, coupled with the potential involvement of CHCs in intraspecific chemical communication, highlight prospective applications in the realms of rapid taxonomic assignment and the targeted management of pest species. As previously illustrated by Potter and Haynes (1993), non-polar solvent extracts derived from female southern masked chafers (*Cyclocephala borealis* Arrow, 1911) trigger sex-specific male behaviour. This intriguing phenomenon can be leveraged in strategies encompassing selective baiting, mass captures, or sexual disruption techniques.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0007485323000664>

**Acknowledgements.** The authors express their gratitude to Paschoal Coelho Grossi for identifying the beetles. Additionally, we extend our thanks to Eduardo Gomes Gonçalves and Rafaella de Lucena Nóbrega for their assistance in collecting *E. emarginata* specimens in Taguatinga and *C. distincta* specimens in Igarassu, respectively. We would also like to acknowledge the invaluable support of Silvio Columbelli and Wesley Nantes during fieldwork in Mato Grosso do Sul. ACDM is sincerely grateful for the hospitality extended by Harri Lorenzi, founder of the Plantarum Institute in Nova Odessa. ACDM and GKNS were partially supported by scholarships from the Coordenação de Aperfeiçoamento de Nível Superior (CAPES). DMAF acknowledges the financial support provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 306351/2022-6) and FACEPE (APQ-0443-



1.06/15). The author(s) received no direct funding to support the research, authorship, and/or publication of this article.

**Competing interests.** None.

## References

- Albuquerque LSCD, Grossi PC and Iannuzzi L (2016) Flight patterns and sex ratio of beetles of the subfamily Dynastinae (Coleoptera, Melolonthidae). *Revista Brasileira de Entomologia* **60**, 248–254.
- Bagnères A-G and Wicker-Thomas C (2010) Introduction: history and overview of insect hydrocarbons. In Blomquist GJ and Bagnères A-G (eds), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. New York, USA: Cambridge University Press, pp. 3–18.
- Barros RP, Astúa D, Grossi PC, Iannuzzi L and Maia ACD (2020) Landmark-based geometric morphometrics as a tool for the characterization of biogeographically isolated populations of the pollinator scarab beetle *Erioscelis emarginata* (Coleoptera: Melolonthidae). *Zoologischer Anzeiger* **288**, 97–102.
- Beach JH (1982) Beetle pollination of *Cyclanthus bipartitus* (Cyclanthaceae). *American Journal of Botany* **69**, 1074–1081.
- Blomquist GJ (2010) Structure and analysis of insect hydrocarbons. In Blomquist GJ and Bagnères A-G (eds), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. New York, USA: Cambridge University Press, pp. 19–34.
- Brückner A and Heethoff M (2017) A chemo-ecologists' practical guide to compositional data analysis. *Chemoecology* **27**, 33–46.
- Caselli A, Favaro R, Petacchi R, Valicenti M and Angeli S (2023) The cuticular hydrocarbons of *Dasineura oleae* show differences between sex, adult age and mating status. *Journal of Chemical Ecology* **49**, 369–383.
- Costa MS, Silva RJ, Paulino-Neto HF and Pereira MJB (2017) Beetle pollination and flowering rhythm of *Annona coriacea* Mart. (Annonaceae) in Brazilian cerrado: behavioral features of its principal pollinators. *PLoS One* **12**, e0171092.
- Dahbi A, Cerdá X, Hefetz A and Lenoir A (1996) Social closure, aggressive behavior, and cuticular hydrocarbon profiles in the polydomous ant *Cataglyphis iberica* (Hymenoptera, Formicidae). *Journal of Chemical Ecology* **22**, 2173–2186.
- El-Sayed AM (2023) The Pherobase: Database of Pheromones and Semiochemicals. Available at <https://www.pherobase.com>
- Endrödi S (1985) *The Dynastinae of the World (Series Entomologica V. 28)*. Hungary: Budapest: Dr W. Junk Publishers.
- Fletcher MT, Allsopp PG, McGrath MJ, Chow S, Gallagher OP, Hull C, Cribb BW, Moore CJ and Kitching W (2008) Diverse cuticular hydrocarbons from Australian canebeetles (Coleoptera: Scarabaeidae). *Australian Journal of Entomology* **47**, 153–159.
- Francis GW and Veland K (1987) Alkylthiolation for the determination of double-bond positions in linear alkenes. *Journal of Chromatography A* **219**, 379–384.
- Garwood RJ and Edgecombe GD (2011) Early terrestrial animals, evolution, and uncertainty. *Evolution: Education and Outreach* **4**, 489–501.
- Gibernau M (2015) Pollination ecology of two *Dieffenbachia* in French Guiana. *Aroideana* **38E**, 38–66.
- Gibernau M and Barabé D (2002) Pollination ecology of *Philodendron squamiferum* (Araceae). *Canadian Journal of Botany* **80**, 316–320.
- Gibernau M, Barabé D, Cerdan P and Dejean A (1999) Beetle pollination of *Philodendron solimoesense* (Araceae) in French Guiana. *International Journal of Plant Sciences* **160**, 1135–1143.
- Gibernau M, Barabé D, Labat D, Cerdan P and Dejean A (2003) Reproductive biology of *Montrichardia arborescens* (Araceae) in French Guiana. *Journal of Tropical Ecology* **19**, 103–107.
- Ginzl MD (2010) Hydrocarbons as contact pheromones of longhorned beetles (Coleoptera: cerambycidae). In Blomquist GJ and Bagnères A-G (eds), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. New York, USA: Cambridge University Press, pp. 375–389.
- Gottsberger G and Silberbauer-Gottsberger I (1991) Olfactory and visual attraction of *Erioscelis emarginata* (Cyclocephalini, Dynastinae) to the inflorescences of *Philodendron selloum* (Araceae). *Biotropica* **23**, 23–28.
- Greene MJ and Gordon DM (2003) Cuticular hydrocarbons inform task decisions. *Nature* **423**, 32–32.
- Hadley NF (1980) Surface waxes and integumentary permeability: lipids deposited on or associated with the surface of terrestrial plants and animals help protect them from a lethal rate of desiccation. *American Scientist* **68**, 546–553.
- Hammer Ø, Harper DA and Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**, 9.
- Hartke J, Sprenger PP, Sahn J, Winterberg H, Orivel J, Baur H, Beuerle T, Schmitt T, Feldmeyer B and Menzel F (2019) Cuticular hydrocarbons as potential mediators of cryptic species divergence in a mutualistic ant association. *Ecology and Evolution* **9**, 9160–9176.
- Henderson A (1986) A review of pollination studies in the Palmae. *The Botanical Review* **52**, 221–259.
- Hirai Y, Akino T, Wakamura S and Arakaki N (2008) Morphological and chemical comparison of males of the white grub beetle *Dasylepida ishigakiensis* (Coleoptera: Scarabaeidae) among four island populations in the Sakishima Islands of Okinawa. *Applied Entomology and Zoology* **43**, 65–72.
- Howard RW and Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology* **50**, 371–393.
- Ingleby FC (2015) Insect cuticular hydrocarbons as dynamic traits in sexual communication. *Insects* **6**, 732–742.
- Jutsum AR, Saunders TS and Cherrett JM (1979) Intraspecific aggression is the leaf-cutting ant *Acromyrmex octospinosus*. *Animal Behavior* **27**, 839–844.
- Kather R and Martin SJ (2012) Cuticular hydrocarbon profiles as a taxonomic tool: advantages, limitations and technical aspects. *Physiological Entomology* **37**, 25–32.
- Lagomarsino LP and Frost LA (2020) The central role of taxonomy in the study of neotropical biodiversity. *Annals of the Missouri Botanical Garden* **105**, 405–421.
- Liang D and Silverman J (2000) “You are what you eat”: diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* **87**, 412–416.
- Lockey KH (1988) Lipids of the insect cuticle: origin, composition and function. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* **89**, 595–645.
- Lockey KH (1991) Insect hydrocarbon classes: implications for chemotaxonomy. *Insect Biochemistry* **21**, 91–97.
- Lyal C, Kirk P, Smith D and Smith R (2008) The value of taxonomy to biodiversity and agriculture. *Biodiversity* **9**, 8–13.
- Maia ACD, Schlindwein C, Navarro DMAF and Gibernau M (2010) Pollination of *Philodendron acutatum* (Araceae) in the Atlantic forest of northeastern Brazil: a single scarab beetle species guarantees high fruit set. *International Journal of Plant Sciences* **171**, 740–748.
- Maia AC, Dötterl S, Kaiser R, Silberbauer-Gottsberger I, Teichert H, Gibernau M, do Amaral Ferraz Navarro DM, Schlindwein C and Gottsberger G (2012) The key role of 4-methyl-5-vinylthiazole in the attraction of scarab beetle pollinators: a unique olfactory floral signal shared by Annonaceae and Araceae. *Journal of Chemical Ecology* **38**, 1072–1080.
- Maia ACD, Gibernau M, Carvalho AT, Gonçalves EG and Schlindwein C (2013) The cowl does not make the monk: scarab beetle pollination of the Neotropical aroid *Taccarum ulei* (Araceae: Spathicarpeae). *Biological Journal of the Linnean Society* **108**, 22–34.
- Maia ACD, Santos GKN, Gonçalves EG, Navarro DMAF and Nuñez-Avellaneda LA (2018) 2-Alkyl-3-methoxypyrazines are potent attractants of florivorous scarabs (Melolonthidae, Cyclocephalini) associated with economically exploitable Neotropical palms (Arecaceae). *Pest Management Science* **74**, 2053–2058.
- Maia ACD, Reis LK, Navarro DM, Aristone F, Colombo CA, Carreño-Barrera J, Nuñez-Avellaneda LA and Santos GK (2020) Chemical ecology of *Cyclocephala forsteri* (Melolonthidae), a threat to macauba oil palm cultivars (*Acrocomia aculeata*, Arecaceae). *Journal of Applied Entomology* **144**, 33–40.
- Moore MR (2011) *Disentangling the phenotypic variation and pollination biology of the Cyclocephala sexpunctata species complex (Coleoptera: Scarabaeidae: Dynastinae)* (Unpublished MSc thesis). Wichita State University.
- Moore MR and Jameson ML (2013) Floral associations of cyclocephaline scarab beetles. *Journal of Insect Science* **13**, 100.

- Moore MR, Cave RD and Branham MA** (2018a) Annotated catalog and bibliography of the cyclocephaline scarab beetles (Coleoptera, Scarabaeidae, Dynastinae, Cyclocephalini). *ZooKeys* **745**, 101–378.
- Moore MR, Cave RD and Branham MA** (2018b) Synopsis of the cyclocephaline scarab beetles (Coleoptera, Scarabaeidae, Dynastinae). *ZooKeys* **745**, 1–99.
- Morel L, Vander Meer RK and Lavine BK** (1988) Ontogeny of nestmate recognition cues in the red carpenter ant (*Camponotus floridanus*). *Behavioral Ecology and Sociobiology* **22**, 175–183.
- Neita-Moreno JC** (2021) A review of the black species of *Cyclocephala* Dejean (Coleoptera: Scarabaeidae: Dynastinae) from Colombia. *Zootaxa* **5026**, 1–58.
- Niogret J, Felix AE, Nicot A and Lumaret JP** (2019) Chemosystematics using cuticular compounds: a powerful tool to separate species in Mediterranean dung beetles (Coleoptera: Geotrupidae). *Journal of Insect Science* **19**, 18.
- Nóbrega RL, Maia ACD, Lima CHM, Felix KES, Souza TB and Pontes WJT** (2022) Behavioral traits and sexual recognition: multiple signaling in the reproductive behavior of *Cyclocephala distincta* (Melolonthidae, Cyclocephalini). *Anais da Academia Brasileira de Ciências* **94**, e20200694.
- Nogueira GAL, Rodrigues SR and Tiago EF** (2013) Biological aspects of *Cyclocephala tucumana* Brethes, 1904 and *Cyclocephala melanocephala* (Fabricius, 1775) (Coleoptera: Scarabaeidae). *Biota Neotropica* **13**, 86–90.
- Núñez Avellaneda LA** (2014) *Patrones de asociación entre insectos polinizadores y palmas silvestres en Colombia con énfasis en palmas de importancia económica* (Unpublished PhD thesis). Universidad Nacional de Colombia.
- Oliveira HN and Ávila CJ** (2011) Ocorrência de *Cyclocephala forsteri* em *Acronomia aculeata*. *Pesquisa Agropecuária Tropical* **41**, 293–295.
- Parizotto DR and Grossi PC** (2019) Revisiting pollinating *Cyclocephala* scarab beetles (Coleoptera: Melolonthidae: Dynastinae) associated with the soursop (*Annona muricata*, Annonaceae). *Neotropical Entomology* **48**, 415–421.
- Potter DA and Haynes KF** (1993) Field-testing pheromone traps for predicting masked chafer (Coleoptera: Scarabaeidae) grub density in golf course turf and home lawns. *Journal of Entomological Science* **28**, 205–212.
- Ratcliffe BC, Cave RD and Cano E** (2013) The dynastine scarab beetles of Mexico, Guatemala, and Belize (Coleoptera: Scarabaeidae). *Bulletin of the University of Nebraska State Museum* **27**, 1–666.
- Ratcliffe BC, Cave RD and Paucar-Cabrera A** (2020) The dynastine scarab beetles of Ecuador (Coleoptera: Scarabaeidae: Dynastinae). *Bulletin of the University of Nebraska State Museum* **32**, 1–586.
- Rodrigues SR, Nogueira GA, Echeverria RR and Oliveira VS** (2010) Aspectos biológicos de *Cyclocephala verticalis* Burmeister (Coleoptera: Scarabaeidae). *Neotropical Entomology* **39**, 15–18.
- Santos MD** (2014) *Revisão do grupo “latericia” do gênero Cyclocephala Dejean, 1821 (Melolonthidae, Dynastinae, Cyclocephalini)* (Unpublished BSc dissertation). Universidade Federal do Paraná.
- Santos V and Ávila CJ** (2007) Aspectos bioecológicos de *Cyclocephala forsteri* Endrodi, 1963 (Coleoptera: Melolonthidae) no estado do Mato Grosso do Sul. *Brazilian Journal of Agriculture* **82**, 298–303.
- Scariot AO, Lleras E and Hay JD** (1991) Reproductive biology of the palm *Acrocomia aculeata* in Central Brazil. *Biotropica* **23**, 12–22.
- Schulz S and Möllerke A** (2022) MACE – an open access data repository of mass spectra for chemical ecology. *Journal of Chemical Ecology* **48**, 589–597.
- Scott MP, Madjid K and Orians CM** (2008) Breeding alters cuticular hydrocarbons and mediates partner recognition by burying beetles. *Animal Behaviour* **76**, 507–513.
- Singer TL** (1998) Roles of hydrocarbons in the recognition systems of insects. *American Zoologist* **38**, 394–405.
- Soon V, Castillo-Cajas RF, Johansson N, Paukkunen J, Rosa P, Ødegaard F, et al.** (2021) Cuticular hydrocarbon profile analyses help clarify the species identity of dry-mounted cuckoo wasps (Hymenoptera: Chrysididae), including type material, and reveal evidence for a cryptic species. *Insect Systematics and Diversity* **5**, 3.
- Souza TB, Maia ACD, Schindwein C, Albuquerque LSC and Iannuzzi L** (2014) The life of *Cyclocephala celata* Dechambre, 1980 (Coleoptera: Scarabaeidae: Dynastinae) in captivity with descriptions of the immature stages. *Journal of Natural History* **48**, 275–283.
- Steiger S and Stöckl J** (2014) The role of sexual selection in the evolution of chemical signals in insects. *Insects* **5**, 423–438.
- Thomas ML and Simmons LW** (2008) Sexual dimorphism in cuticular hydrocarbons of the Australian field cricket *Teleogryllus oceanicus* (Orthoptera: Gryllidae). *Journal of Insect Physiology* **54**, 1081–1089.
- van Den Dool H and Kratz PD** (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography* **11**, 463–471.
- van Zweden JS and d’Ettorre P** (2010) The role of hydrocarbons in nestmate recognition. In Blomquist GJ and Bagnères A-G (eds), *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. New York, USA: Cambridge University Press, pp. 222–243.
- Wagner D, Brown MJ, Broun P, Cuevas W, Moses LE, Chao DL and Gordon DM** (1998) Task-related differences in the cuticular hydrocarbon composition of harvester ants, *Pogonomyrmex barbatus*. *Journal of Chemical Ecology* **24**, 2021–2037.
- Young HJ** (1986) Beetle pollination of *Dieffenbachia longispatha* (Araceae). *American Journal of Botany* **73**, 931–944.