



Camouflaged Nests of *Mischocyttarus mirificus* (Hymenoptera, Vespidae)

Lucas Rocha Milani¹ · Rafael Alves Bonfim de Queiroz² · Marcos Magalhães de Souza³ · Mateus Aparecido Clemente⁴ · Fábio Prezoto¹

Received: 3 September 2020 / Accepted: 30 August 2021
© Sociedade Entomológica do Brasil 2021

Abstract

Social wasps present various architectural patterns for their nests, which may differ in shape, size, color, and material used in construction. The distribution pattern of comb cells presented by *Mischocyttarus mirificus* (Zikán, 1935) is vertical with a single cell of width, resulting in a filiform shape that camouflages in the middle of the substrate. There are few studies regarding this architectural pattern for social wasps and their role in camouflage, and this study aims to detail the nesting habits of this species. In total, 40 colonies were analyzed in their natural habitat and six were dissected in the laboratory. Aspects about the construction of the nests and nesting environment have been described. A test was carried out with nest photographs, to quantify their camouflage percentage within the nesting substrate. The colonies were found mainly in riparian forests where there were elements in the environment that favored their camouflage. According to the nest photograph analysis, their camouflage levels can vary according to the position from which the photograph is taken. It is evident that *M. mirificus* founders select sites where they can camouflage their colonies, and this gives them advantages in exploring a new environment.

Keywords Architectural · Colony · Environment · Mischocyttarini · Social wasps

Introduction

The genus *Mischocyttarus* de Saussure, 1853 is the only one that represents the Mischocyttarini tribe, comprising of approximately 250 described species (Silveira 2008). The colonies of the genus consist of a single comb without protective covering and fixed to the substrate by a peduncle

(Wenzel 1998; Somavilla et al 2012). The species are essentially neotropical, distributed exclusively in Central and South America, and with a few species occurring in Northern Mexico (Richards 1978, Carpenter and Andena 2013).

Mischocyttarus mirificus (Zikán) has been recorded in Southeastern Brazil in Parque Nacional do Itatiaia (RJ) and in Área de Proteção Ambiental (APA) São José (MG) (Barbosa et al 2016a; Souza et al 2020a). *Mischocyttarus mirificus* is part of the *Haplometrobius* subgenus and the *Mischocyttarus artifex* (Ducke, 1914) species group. These groups have various architectural patterns, and some species, such as *Mischocyttarus artifex*, *M. mirificus*, *Mischocyttarus oecothrix* Richards, 1940, and *Mischocyttarus ypiranguensis* Fonseca, 1926, build string-like combs, with a single cell of width (Richards 1978).

The filiform architecture pattern presented by *M. mirificus* nests is a subject of curiosity because, when in their natural environment, they are often mistaken for branches, vines, and roots of epiphytic plants (Souza et al 2010a). However, studies that aimed to investigate aspects related to the camouflage behavior of nests in social wasps are scarce (Turillazzi 2012; Barbosa et al 2016b; Milani et al 2020;

Edited by Stefano Colazza

✉ Lucas Rocha Milani
lucassmilani@gmail.com

- ¹ Depto de Zoologia, Instituto de Ciências Biológicas, Programa de Pós Graduação em Comportamento E Biologia Animal, Univ Federal de Juiz de Fora, Minas Gerais, Juiz de Fora, Brazil
- ² Depto de Ciência da Computação, Instituto de Ciências Exatas, Univ Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil
- ³ Instituto Federal de Educação, Ciência e Tecnologia, Sul de Minas Gerais, Inconfidentes, MG, Brazil
- ⁴ Depto de Zoologia, Univ Estadual Paulista “Júlio de Mesquita Filho”, Rio Claro, São Paulo, Brazil

Souza et al 2020b), while a few others address them briefly (Richards 1978; Giannotti 1999; Smith 2004; Starr 2014). Camouflage, much like nesting site selection, is considered to be a primary defense strategy (Edmunds 1974), as it is an evolutionary adaptation found in some social wasp species (Hermann and Blum 1981; Souza et al 2020b), especially in those which present low aggressiveness (Strassmann et al 1990) or stunted stingers, like *Mischocyttarus* (Giannotti 1999; Silveira et al 2015).

Richards (1978) described the architectural patterns of many *Mischocyttarus* species' colonies and provided insights into their possible camouflage potential for many of them. Giannotti (1999) wrote that colonies of *Mischocyttarus cerberus styx* Richards, 1940 were well camouflaged within their substrate, since their coloration resembled the bark of the trees on which they were found; additionally, he described the behavior of adult wasps covering the nest's naturally white pupae cells with plant material so that they do not contrast against the background. Another report pointed out that the nests of *M. collarellus* are extremely cryptic: not only were they about the same stained color as the trunk of the tree they were on, but they were also similar in shape and size to a hanging tree bark piece (Smith 2004). It is also suggested that the elongated and filiform shape of the *Mischocyttarus punctatus* (Ducke, 1904) nests (similar to the pattern of *M. mirificus*) consists of a defensive camouflage strategy (Starr 2014).

Animals capable of camouflaging themselves are under strong pressure to minimize their conspicuity to possible predators, and the efficiency of this behavior is related to the mechanisms used by the predator in the search for prey (Pike 2018). A more thorough investigation of the aspects involved in the camouflage behavior of the *M. mirificus* nests will assist in the better understanding of its evolutionary and adaptive meaning. And if the colony's camouflage is successful, this may result in *M. mirificus* having equal or superior productivity when compared to the other species of the genus. Thus, the aim of this study was to measure the productivity of *M. mirificus* colonies, describe their nesting habits, and provide a new strategy to quantify nest camouflage.

Material and methods

Study area

The study was carried out at riverbank forest fragments in the municipalities of Inconfidentes (22°19'1"S, 46°19'40"W) and Bueno Brandão (22°26'27"S, 46°21'3"W), south of Minas Gerais state, Brazil, under the influence of the Atlantic semi-deciduous montane forest phytophysiology (Oliveira Filho 2006), where colonies of the species have

typically been found. Both areas present warm climate, with an average of 17.3 and 19.3 °C for Bueno Brandão and Inconfidentes respectively, according to Koppen and Geiger (Climate-date 2018). Field visits were conducted monthly from 12:00 p.m. to 5:00 p.m. from March 2017 to February 2018, totaling 15 days of fieldwork and 75 h of sampling divided equally between locations.

Data collection

The riverbank forest areas of the two municipalities were actively searched to locate colonies, which involved visual searches for wasp colonies by multiple observers, ranging from two to five samplers. The searches were carried out on pre-existing trails for an unmarked distance with varied proximity to water bodies.

When found, colonies were photographed and measurements of nest length, cell number, number of adults, distance from the nearest water source, distance from the ground, substrate used for nesting, and color of the nest peduncle were recorded. When nests were found abandoned (absence of adults, eggs, larvae, and pupae), they were collected for laboratory analysis.

Individual wasps were sampled and sent to Professor Orlando Tobias da Silveira at Museu Emílio Goeldi to confirm species identification. The specimens were deposited in the CBVS collection speciesLink system, IFSULDEMINAS Zoology Laboratory, Campus Inconfidentes, by the catalog numbers: 00708–2014; 00,709–2014; 00,710–2014; 03,446–2017; 03,447–2017; 03,448–2017; 03,449–2017; 03,449–2017.

Colony productivity

The nests were taken to Laboratório de Ecologia Comportamental e Bioacústica (LABEC) at Universidade Federal de Juiz de Fora, where some of them were dissected to characterize nest architecture and infer productivity indexes. Additionally, the following data was recorded from them: cell length, productive cells, number of adult wasps emerged (calculated by counting the meconium layers inside the cells), number of reused cells, and the length and width of the nest peduncle (Oliveira et al 2010).

First, peduncle length and width were measured with calipers. Later, each cell was analyzed individually for length and width. Finally, through dissection under the stereomicroscope, it was possible to assess the amount and width of the meconium layers in each cell.

Data analysis

The Spearman correlation coefficient was used between peduncle width and cell number, total cell number and

number of adults produced by the colony, and number of meconium layers and the length of the cells. Pearson's correlation was used between colony length and cell number, with a 5% confidence margin. Both statistical analyses were carried out in the R Software (R Development Core Team 2017).

Strategy used to quantify nest camouflage

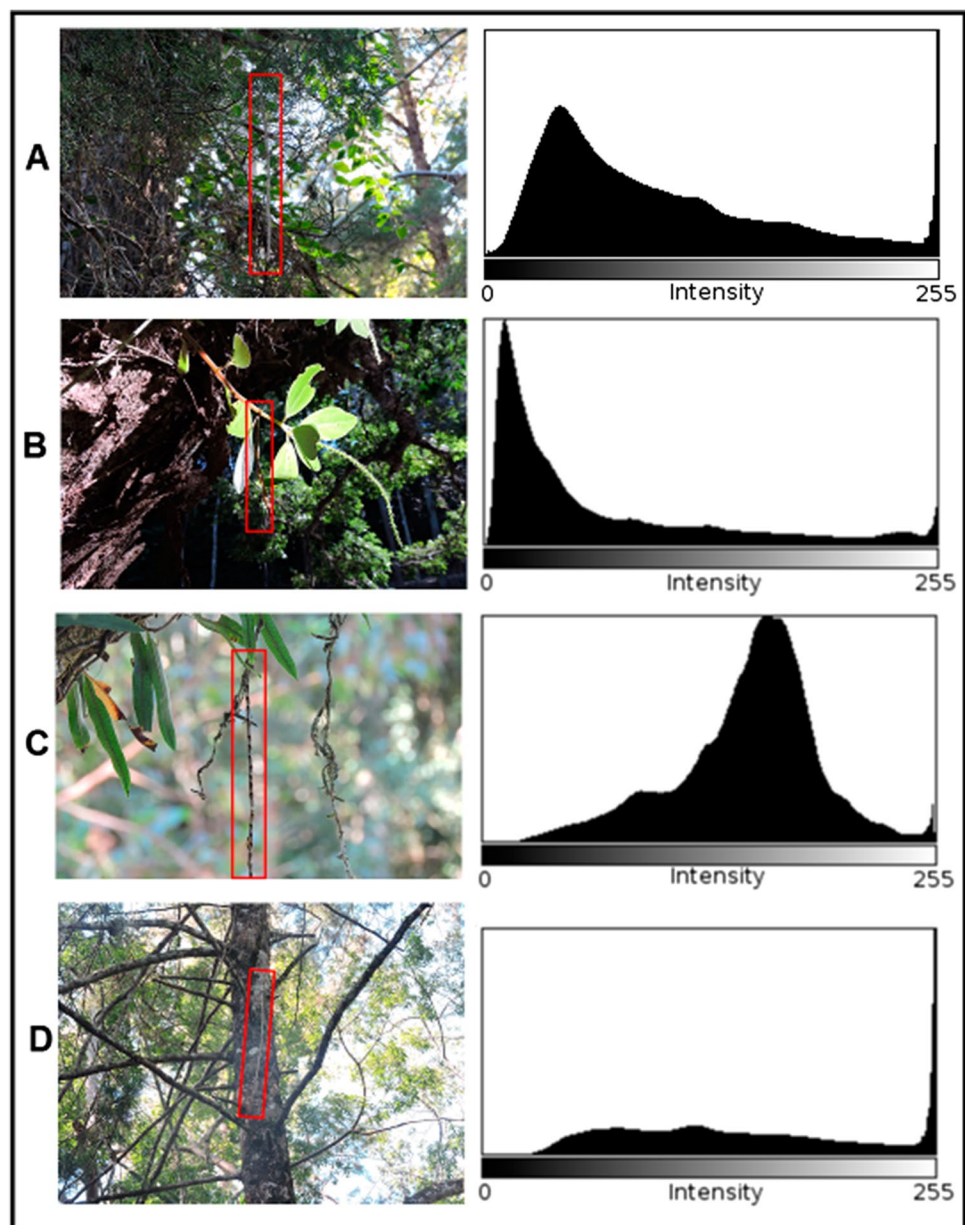
Despite the difficulties of photographing in the field, eight *M. mirificus* colonies were found and photographed from at least two different perspectives/positions.

The resolution of the photos is 4608×3456 pixels, taken with a NIKON® camera (model: COOLPIX P600). Figure 1

shows examples of photos taken of colonies. In this figure, the histograms of the images were obtained using the free, open-source image analysis software ImageJ (Schneider et al 2012) with weighted RGB conversions.

The bias in the acquisition of images was avoided through the following procedures: (i) illumination settings such as “skylight” and “cloudy” were not used on the digital camera; (ii) the camera flash was disabled to maintain natural illumination behavior (e.g., shadows); (iii) the photos of the colonies were taken at a distance that allowed the view of the entire nest and from different perspectives (back, front, left, and right) in order to avoid any experimenter bias in target placement; and (iv) the photos were taken to include

Fig. 1 Photographs of *Mischocyttarus mirificus* colonies labeled as A, B, C, and D with their respective histograms. The y-axis of the histogram represents the frequency of occurrence of each gray-level value. Red rectangle highlights the colonies



the landscape where the colony is inserted, with surrounding branches, roots, and epiphytes.

The threshold color plugin of ImageJ, which is based on a bandpass filter, was used to analyze the colored photos of the *M. mirificus* colonies (Schneider et al 2012). It can threshold color images in the RGB system in different color spaces, such as HSB, which was the one chosen for this study. The HSB space has three components that define the color of each pixel: hue (H), saturation (S), and brightness (B). The RGB space defines the pixel color through three components: red (R), green (G), and blue (B). Details about color spaces and conversions between them can be seen in Gonzalez and Woods (2009).

The intensity of a pixel or a region of interest (ROI) formed by a set of pixels serves as the input data for the threshold color plugin. This input data is provided to the plugin through ImageJ's graphical interface. As the focus in this study is the wasp nest, each plugin application was provided with a rectangular domain within the nest space as the ROI.

Figure 2 illustrates the ROI chosen to be the input for the threshold color plugin. This region is determined via ImageJ graphical interface using a mouse click event by the user. After the user chooses the ROI, the filter determines regions within the image that have colors close to those contained in the ROI.

The color segmentation plugin (Sage 2018) was also used to quantify the painted area obtained with the application of the threshold color plugin. It was added to ImageJ software to determine the area of the regions present in an image using the *K*-means algorithm clustering method. The input data of this plugin are points in the form of color keys assigned to regions of the image whose areas are sought.



Fig. 2 Sample image used to perform the camouflage analysis. The arrow indicates a rectangular ROI (edges in yellow) chosen within the *Mischocyttarus mirificus* colony area

Four metrics based on results obtained using the threshold color and color segmentation plugins are proposed in this work to quantify nest camouflage, namely:

- metric A1: percentage of the area of the colony within the image. To calculate this metric, first, the user manually paints the colony area in the image using the ImageJ software. Then, the color segmentation plugin is used to calculate the area of the painted region. Figure 3 (column c2) shows examples of images with the colony area painted by the user;
- metric A2: percentage of the area painted via threshold color plugin within the image. Again, the color segmentation plugin is adopted to calculate this percentage. Figure 3 (column c3) illustrates the area painted using the threshold color plugin;
- metric A3: percentage of the area painted via threshold color plugin within the image considering only the colony area defined by the user. To obtain this percentage, an algorithm developed in this work identifies only the region of the colony that was painted by threshold color plugin. The data input of this algorithm are the images used to calculate the metrics A1 and A2 (e.g., Fig. 3 — columns c2 and c3). It was implemented computationally in the GNU Octave programming environment. Figure 3 (column c4) shows examples of images of the colony region obtained by algorithm whose percentage of area within the image determines metric A3;
- metric M1: ratio between the values of the metrics A2 and A1, i.e., percentage of the area painted using the threshold color plugin (metric A2) divided by percentage of the colony area painted by user within the image (metric A1). Metric M1 suggests the level of wasp colony camouflage;
- metric M2: ratio between the values of the metrics A3 and A1. This metric may be used as a measure of the quality of the ROI for the application of the threshold color plugin.

Results

Nesting habitats

Forty *Mischocyttarus mirificus* colonies were recorded; all fixed on vegetal substrates. This included aerial roots of different epiphytes species, as well as stems or leaves. About 92% of the colonies found were within 15 m of some water body, usually in riparian forests as shown in Fig. 4.

The nests varied in length and cell number; however, even though some of them varied considerably in these aspects, the length correlated positively with the cell number ($r = 0.99$, $p = 2.2e^{-16}$).

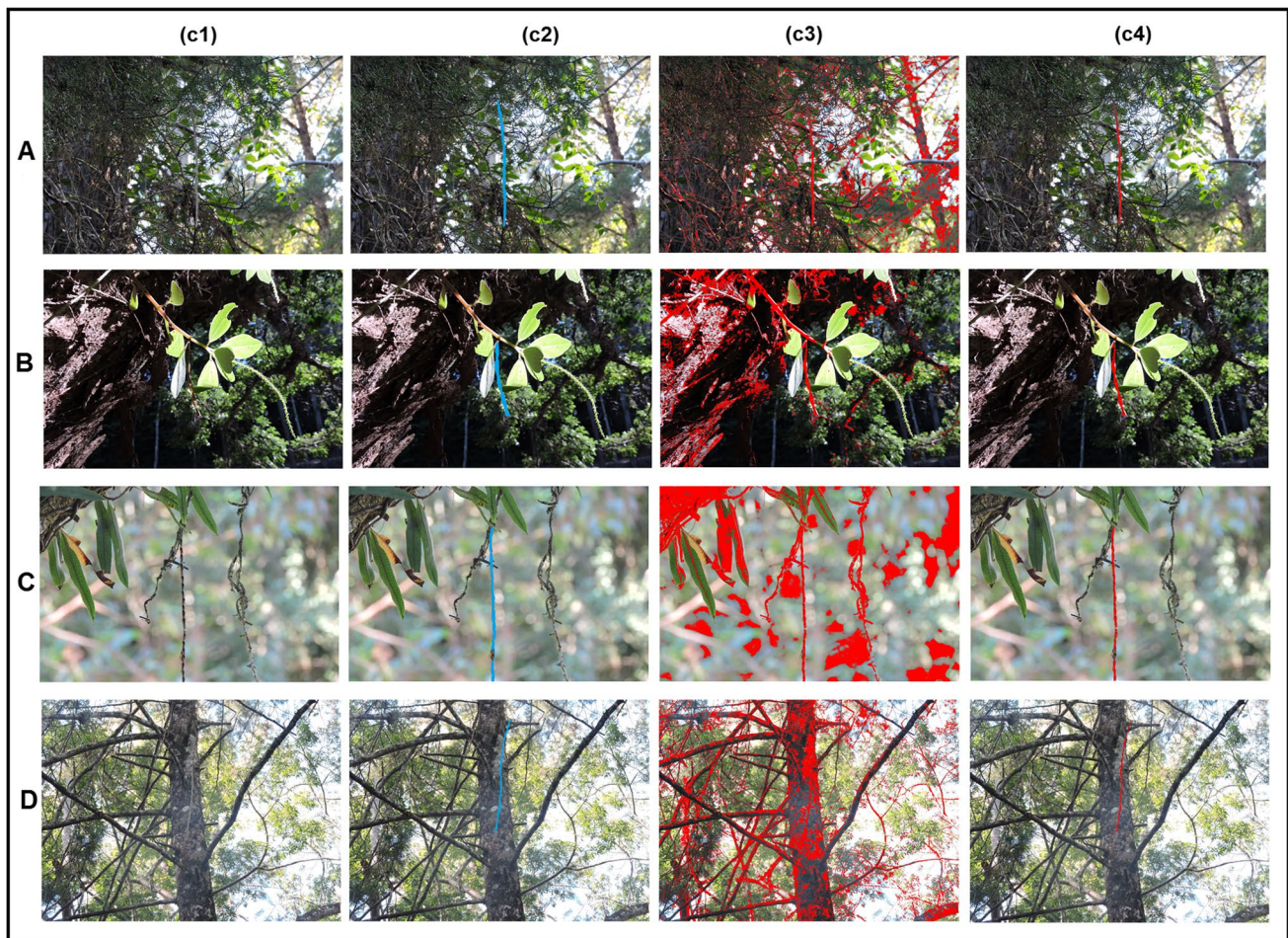


Fig. 3 Photographs of *Mischocyttarus mirificus* colonies labeled as A, B, C, and D. (c1) Original photos, (c2) nest manually highlighted in blue in the photo (c3) results of the application of the threshold color

plugin, (c4) identification of the nest only within the images shown in column c3

The smallest colonies recorded in the field had five cells, while the largest of them presented a total of 103 cells. An average of 6.09 ± 5.66 adults per colony was recorded. The mean distance from the nest to the nearest water body was $5.35 \text{ m} \pm 2.77 \text{ m}$, except for two nests that were approximately 600 m from any body of water. Regarding distance from the ground, an average of $3.46 \pm 1.81 \text{ m}$ was obtained. Ninety-five percent ($n=38$) of the colonies observed were between the heights of 1.5 m and 5 m.

Nest building

Peduncle

The substrates used for nest fixation are preferably horizontally inclined. Initially, a resinous peduncle, which is fixed centrally in the first cell, is built in a vertical or slightly inclined position. The peduncles maintain a similar width to its initial state, slightly widening as the

cell number increases but with no significant correlation ($r=0.58$, $p=0.9$). The analyzed nest peduncles had a mean length of $4.97 \pm 2.75 \text{ mm}$ and a mean width of $1.57 \pm 0.71 \text{ mm}$. All recorded peduncles were shiny black or bright gray in color.

Cell construction

Once the peduncle is completed, the wasps begin the construction of the first cell, which has a conical shape, like the rest of the nest. Initially, the cell is symmetrical in the length of its frontal and dorsal surfaces. As it is enlarged by workers, the dorsal surface becomes slightly larger than the frontal one, causing the opening to be inclined. Because of the vertical-filiform disposition of cells of this architectural pattern, the upper cell needs to be at least partially constructed before the lower one can be initiated. The initial cell, which has not yet originated any adult and is considered incomplete, is relatively smaller than a complete cell, which

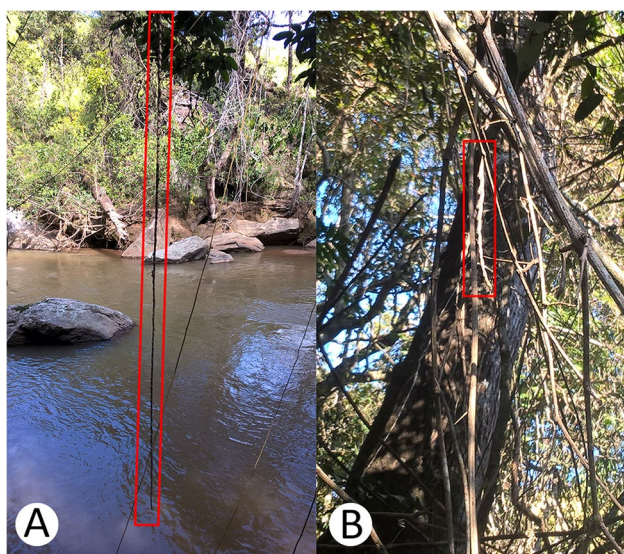


Fig. 4 Colonies of *Mischocyttarus mirificus* in their natural environment. **A** Red rectangle highlights the colony of *Mischocyttarus mirificus* amidst the vines. **B** Red rectangle highlights the colony of *Mischocyttarus mirificus* among dry branches

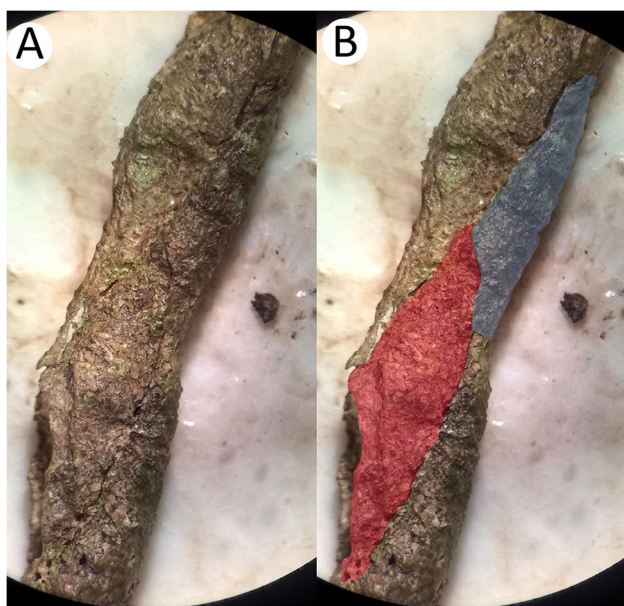


Fig. 5 Detail of a nest cell. **A** Record of a *Mischocyttarus mirificus* nest part in stereomicroscope (45 \times). **B** The blue color represents the initial cell size while the red color represents the increase in cell size. Both colors together represent the final cell size

has already originated at least one adult (see Fig. 5). Cell length correlated positively with the amount of meconium layers found per cell ($r=0.96$, $p=0.008$).

As the nest increases in cell number, the wasps perform small adjustments so that the cell row remains in the same spatial position, thus avoiding nest curvature. Such corrections are made to cells that were initially skewed to either side of its upper neighbor. However, in some nests with many cells, this curvature may still exist, leaving them with a semi-spiral appearance.

Colony productivity

Six nests were dissected (L1 to L6) and their general data is presented in Tables 1 and 2. The mean number of cells per *M. mirificus* nests was 47.66 ± 14.76 ; the mean percentage of productive cells was 79.3 ± 15.36 ; and the mean number of adults produced per colony was 54.16 ± 17.22 . The mean number of individuals produced per cell was 0.93 ± 0.24 . There was a positive correlation between the total number of cells in the colonies and the number of emerged adults ($r=0.93$, $p=0.0005$).

The most productive *M. mirificus* cells were located near the initial cell, in the oldest region of the comb. The less productive cells, on the other hand, were concentrated from the middle to the end of the nest, the youngest part of the comb.

Analysis of nest camouflage

The results obtained with the computational strategy are provided here. The photos of the *M. mirificus* colonies considered in this analysis are labeled nests A, B, C, D, E, F, G, and H (Table 3). In this analysis, the dissected colonies (Table 1) were not included.

Since the nests A, B, C, D, E, F, G, and H were photographed from at least two different perspectives, each nest was renamed using the nest label and the view in which it was recorded by the photographer. The letters b, f, l, and r indicate the “back,” “front,” “left,” and “right” views, respectively. For example, the nest labeled as Dr denotes the right view of nest D.

In Table 3, the results of the metrics A1, A2, A3, M1, and M2 are shown. According to metric M1, regarding level of camouflage, the nests labeled as Dr, El, Ef, and Gf have a higher camouflage level. On the other hand, the nests labeled as Cr, Fl, and Gl presented low values in this metric, which suggests that under these views (r or l), the nests C, F, and G have a low camouflage level.

From Table 3, the mean and standard deviation with the results of the metrics M1 and M2 are calculated for each nest and for the combination of all nests. These results are shown in Fig. 6. The graph associated with metric M1 indicates that nests A, B, and C are less camouflaged than the other nests. On the other hand, nests D and E have a high level of camouflage when compared to the average value of metric M1 obtained considering all nests. The graph related to metric M2 corroborates that the ROI chosen in each nest for applying the threshold color plugin is adequate, as it represented the color pattern of the nest in most cases.

Table 1 Comparative data per colony on the productivity of 6 dissected colonies of *Mischocyttarus mirificus* (L1 to L6). *CN*, total cell number; *NCU*, number of cell uses — 1, one use; 2, two uses; 3, three uses; % *PC*, average percentile rate of productive cells; *PA*, produced adults; *PA/CN*, produced adults by cell number

Colonies	CN	NCU			% PC	PA	PA/CN
		1	2	3			
L1	35	20	7	0	80	34	0.97
L2	56	34	11	5	89.3	71	1.26
L3	62	43	4	0	77.5	51	0.82
L4	54	16	11	0	50	38	0.7
L5	55	28	11	9	87.3	77	1.4
L6	24	15	6	7	91.7	54	2.25
Average	47.66				79.3	54.16	0.93
Standard deviation	14.76				15.36	17.22	0.24

Table 2 Height and width of cells from 6 nests (mean, standard deviation, and variation) in relation to the meconium layers found deposited at the bottom of each cell of the *Mischocyttarus mirificus*

colonies ($n=230$). *NML*, number of meconium layers; *CN*, total cell number; *LL*, layer length in mm; *CL*, cell length in mm; *CW*, cell width in mm

NML	CN	LL mm	CL mm	CW mm
1	156	2.03 ± 0.38 (1.04–2.51)	15.9 ± 1.6 (9.8–20)	2.95 ± 0.14 (2.7–3.2)
2	50	1.32 ± 0.33 (0.85–1.8)	17.2 ± 1.2 (15.3–20)	3.04 ± 0.08 (2.9–3.3)
3	24	1.02 ± 0.09 (0.92–1.1)	18.0 ± 0.9 (16.1–19.9)	3.06 ± 0.06 (2.9–3.1)

Table 3 Quantification of measurements through image analysis of *Mischocyttarus mirificus* nests. Perspective = Nest label + view from which it was recorded in the photograph. The letters b, f, l, and r indicate the “back,” “front,” “left,” and “right” views, respectively

Nest	Perspective	Metric A1	Metric A2	Metric A3	Metric M1	Metric M2
A	Back (Ab)	0.54	10.71	0.475	19.83	0.88
	Front (Af)	0.7	19.23	0.679	27.47	0.97
B	Front (Bf)	0.62	10.36	0.577	16.71	0.93
	Left (Bl)	1.04	22.29	0.946	21.43	0.91
C	Front (Cf)	0.65	21.4	0.643	32.92	0.99
	Right (Cr)	1.52	20.75	1.201	13.65	0.79
D	Front (Df)	0.28	16.67	0.272	59.54	0.97
	Right (Dr)	0.17	20.68	0.163	121.64	0.96
E	Left (El)	0.15	24.31	0.147	162.07	0.98
	Front (Ef)	0.07	13.22	0.061	188.86	0.87
F	Left (Fl)	1.08	13.42	1.037	12.43	0.96
	Right (Fr)	0.71	37.28	0.703	52.51	0.99
G	Front (Gf)	0.31	28.09	0.254	90.61	0.82
	Left (Gl)	1.19	15.62	1.131	13.13	0.95
H	Front (Hf)	0.67	17.48	0.516	26.09	0.77
	Left (Hl)	0.56	34.76	0.291	62.07	0.52

Discussion

Nesting habitats

Mischocyttarus mirificus nests were mostly found in riparian forests, with elements in the environment that resembled their architecture, such as vines, branches, or epiphytic roots, favoring their camouflage. Riparian forests offer a great complexity in vegetal structures, which

can favor social wasps since it provides great variety of physical supports for the colony, increases the amount and heterogeneity in food resources, and imposes less microclimate variability (Lawton 1983; Souza et al 2010b). This has also been reported for *Mischocyttarus flavitarsis* (Saussure, 1854), which mainly occurs in riparian forests (Little 1979).

The nesting substrates used by *M. mirificus* were quite diverse, and there was no obvious preference for a plant species. As already reported in another study, social wasps do

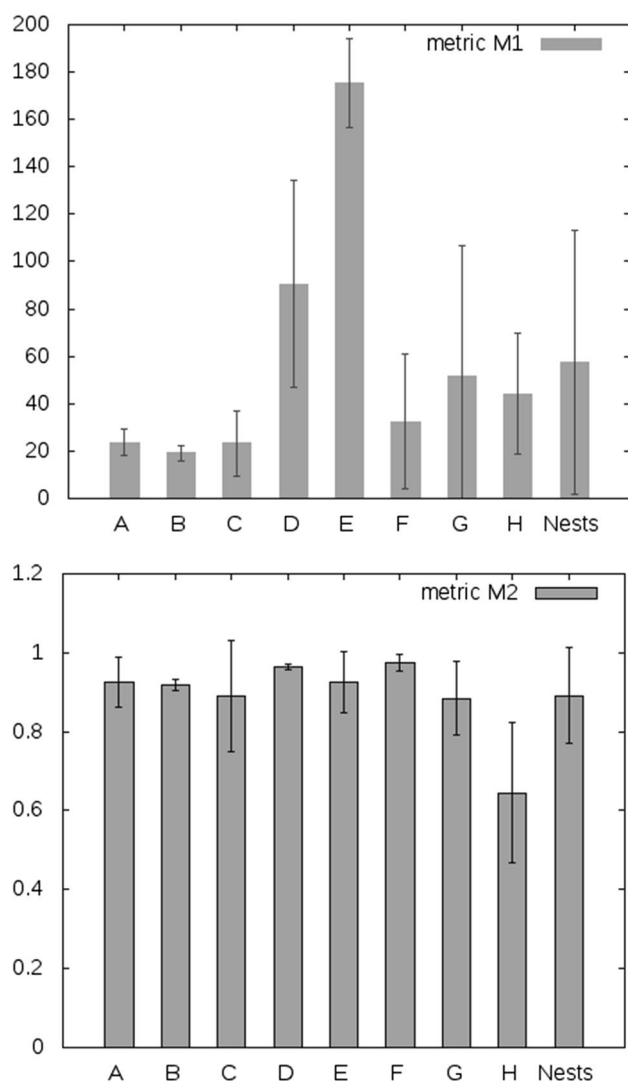


Fig. 6 Average and standard deviation of the values of the metrics M1 and M2. Values of the average (bar) and standard deviation (error bar) of the metrics M1 and M2 are from Table 3. The label “Nests” indicates that the results of the metrics M1 and M2 are regarding all nests combined (nests A to H)

not seem to be influenced by a particular species of plant, but rather by the complexity generated by the heterogeneity of the environment (Souza et al 2014; Francisco et al 2018). When nesting, they seem to look for certain aspects and factors that can be found in many substrates, such as protection and availability of food and water resources (Santos et al 2009).

In a different study, the species *Mischocyttarus collarellus* Richards, 1940 showed a preference for nesting in guava trees (*Psidium* sp.), even with the availability of different potential sites in the area (Smith 2004). It was also observed that all the substrates used by *M. collarellus* had a characteristic in common: the presence of few epiphytes. The author assumes that the epiphytes may be a means for predators to

access the colonies (Smith 2004). However, this does not align with the present study, in which *M. mirificus* colonies were generally found amidst those plants, which we believe is a colony camouflage method.

Mischocyttarus colonies usually do not present large populations, which are usually less than 30 individuals (Giannotti and Trevisoli 1993), and the average population found in the present study was similar to those described in other studies (Torres et al 2011; Giannotti 1999). The same was true for the distance of the colony from the ground (Smith 2004; Castro et al 2014). As for the number of cells presented by *M. mirificus* nests, in contrast to what was suggested by Robert L. Jeanne in 1975, half of the colonies found had 24 cells or more, with the largest of the nests reaching 103 cells and with a colony cycle that extended for more than a year (Souza, M.M personal communication, 2018).

Nest building

Peduncle

The mean length and width of the peduncle were close to that found by some authors for other species of the genus (Giannotti 1999; Montagna et al 2010; Scobie and Starr 2012). In the present study, the peduncle was always central, and its increasing width was not significantly correlated with the growth of cell numbers, contrary to records for other species (Giannotti 1999, Montagna et al 2010).

Cell construction

Commonly in the colonies of social wasps, the cells are initially rounded and, as neighbor cells are added, they assume their characteristic hexagonal shape. This can be observed in species with nests whose cells are hexagonal at the comb's center and rounded on the comb's periphery, especially on the edges (Wenzel 1998; Oliveira et al 2010). Notwithstanding, the pattern presented by *M. mirificus* cells always maintains a rounded or oval shape, as they do not share their side walls.

The internal cell space tends to decrease as each new adult emerges, as just before they become pupae, the larvae release all their excreta, which accumulate at the bottom of the cells, giving rise to the meconium layers, which reduce the internal space of the cell. Thus, as the pupa develops and grows, adjustments are made in the form of expansion of cell walls to properly house the pupal cocoon, which increases cell length. This aligns with what was previously recorded for *M. cerberus styx* (Giannotti 1999).

Some disadvantages to this pattern are cited in literature. Because this disposition does not allow cells to share their side walls, a greater amount of material and energy is required to build each new cell, which is why nests with 25

cells or less are expected (Jeanne 1975). Elongated nests also affect social life, reducing interactions among individuals, and making it harder for the dominant female to maintain a reproductive monopoly (Starr 1991, 2014).

However, even considering the disadvantages, researchers assume that the filiform nest shape must be related to its camouflage and could be somewhat advantageous (Jeanne 1975; Starr 1991, 2014). Other possible advantages of filiform nests could be a lower probability of being knocked over by birds at once, which may happen to more compact nests, and a lower vulnerability to being totally eliminated by parasites migrating from cell to cell (Herre et al. 1986).

Colony productivity

The mean number of productive cells in *M. mirificus* nests was close to that found in other studies (Giannotti 1998, Penna et al. 2007a, b, Montagna et al 2010, Castro et al 2014). However, *M. mirificus* had a higher mean number of productive cells than *Mischocyttarus cassununga* (Castro et al 2014) and *Mischocyttarus consimilis* (Montagna et al 2010).

Mischocyttarus mirificus nests seemed to produce fewer cells when compared to other species of the genus. However, it showed a higher average of adults produced, as well as number of adults emerged per cell. The number of uses of each cell was also very close to those recorded by other researchers. Reusing the cells is a way for social wasps to save energy and material for the construction of new cells, which can be costly in this architectural pattern. These differences may be related to the intrinsic biological differences between species and/or the environments in which studies are conducted, making comparisons a complicated effort (Castro et al 2014).

Some authors suggest that the most productive cells in colonies of *Mischocyttarus* and *Polistes* are the central ones (horizontal shaped colonies), since they probably receive greater parental care from the females due to more frequent monitoring (Giannotti and Machado 1999) and because it is a possible strategy against predation and parasitism (Gobbi et al 1993), additionally to being the oldest cells of the colony (Castro et al 2014). The same can occur with *M. mirificus* with the cells closest to the peduncle (upper region, considering the filiform shape of the colony), which we found to be the most productive region of the colony.

Analysis of nest camouflage

As previously mentioned, the nests of *M. mirificus* resemble the substrate on which they are inserted, requiring careful observation to be found. The shape assumed by the vertical and linear cell distribution is easily mistaken by the substrate when observed. However, nests resemble the substrate not

only in shape, but also in coloration. This can be observed applying the threshold color plugin from ImageJ software with a ROI defined within the *M. mirificus* colony. Figure 3 (column c3) shows the results of this plugin.

Turillazzi (2012) discusses architectural patterns of several species of the Stenogastrinae subfamily, many of which resemble the pattern of *M. mirificus*. Several of the colonies observed by the author closely resemble the substrate on which they are inserted in characteristics such as size, shape, and color. In the same study, Turillazzi states that one of the main defenses of the Stenogastrinae colonies is camouflage, knowing that hornet wasps are its main predators and that they use visual cues to find their prey's colonies. This suggests that the main selection force which led *M. mirificus* to present this architectural pattern may have been a predator that uses such cues when foraging.

A very fortuitous event, which can further testify to the nest camouflage capability, was that during one of the field observations, a damselfly from the Suborder Zygoptera landed in the colony to rest and only took flight when it was attacked by a wasp. Knowing that dragonflies see very well and use mainly vision to orient themselves (Olberg et al 2005), the fact that the individual did not perceive the difference between the colony and the branches is worth noting.

The coloration of the *M. mirificus* colonies contributes to camouflage, according to the metrics A2 and M1 from Table 3. This suggests that the plant material used in the nest construction is taken from the places where the colonies are found, as observed for *M. cerberus styx* (Giannotti 1999). Furthermore, Table 3 shows that the perspectives influence the results of the metrics. Thus, some positions in which the colonies are photographed can attest a higher level of camouflage than others due to the incidence of light through the camera lens. The same can occur with possible *M. mirificus* predators guided by visual cues.

Despite a limited number of images of *M. mirificus* colonies, they suggest that colors present in the comb contribute to their level of visual camouflage.

The preliminary results obtained using the computational strategy proposed to quantify the nest camouflage demonstrate that this approach is promising and can further be applied for other social wasps.

Mischocyttarus mirificus founders look for environments and nesting substrates not based on a preference for a particular plant substrate, but on aspects present in several plant species that allow the colony to be camouflaged. Further studies are needed to assess which aspects selected this architectural pattern in the nests of *M. mirificus*.

Acknowledgements We are grateful to Dra. Nádia Sílvia Somavilla from Instituto de Ciências Biológicas, Departamento de Botânica. We are also grateful to all who assisted with the field work.

Author contribution Lucas Rocha Milani — The main author, responsible for conducting all phases of this study. He carried out data collection, statistical analyses, writing and revision of the manuscript, and presentation of results and discussion.

Rafael Alves Bonfim Queiroz — The author was responsible for the application of the technology used in this study. Additionally, he collaborated in writing, analyzing the data, and interpreting it.

Marcos Magalhães de Souza — The author collaborated with data collection, data analysis and writing of this study, oversight and leadership responsibility for the research activity planning and execution, computing resources and other analysis tools, planning and execution, design, conducting the experiments, analyzing and interpreting the data, and writing the manuscript.

Mateus Aparecido Clemente — The author collaborated writing this study, analyzing the data, and presenting the results.

Fábio Prezoto — Responsible for management and coordination of research activity, provision of study materials, reagents, sampling and analysis materials, laboratory samples, oversight and leadership regarding research activity planning and execution, computing resources and other analysis tools, planning and execution, design, conducting the experiments, analyzing and interpreting the data, and writing the manuscript.

Funding We acknowledge the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) and Coordenação de Aperfeiçoamento Pessoal de Nível Superior—Brasil (CAPES)—Finance code 001 for their financial support.

Declarations

Conflict of interest The authors declare no competing interests.

References

- Barbosa BC, Detoni M, Maciel TT, Prezoto F (2016) Studies of social wasp diversity in Brazil: over 30 years of research, advancements and priorities. *Sociobiology* 63(3):858–880. <https://doi.org/10.13102/sociobiology.v63i3.1031>
- Barbosa BC, Dias LM, Vieira KM, Prezoto F (2016b) Cryptic nest of *Mischocyttarus iheringi* (Hymenoptera: Vespidae). *Florida Entomologist* 9(1):135–138. <https://doi.org/10.1653/024.099.0130>
- Carpenter JM, Andena SR (2013) The vespidae of Brazil. Manaus, Instituto Nacional de Pesquisa da Amazônia, Manaus, Brazil
- Carpenter JM, Marques OM (2001) Contribuição ao Estudo dos Vespídeos do Brasil. Cruz das Almas, Universidade Federal da Bahia, Série Publicações Digitais 3: CD-ROM
- Castro MM, Avelar DLG, Souza AR, Prezoto F (2014) Nesting substrate, colony success and productivity of the wasp *Mischocyttarus cassununga*. *Revista Brasileira de Entomologia* 58(2):168–172. <https://doi.org/10.1590/S0085-56262014000200009>
- CLIMATE-DATE (2018) <https://pt.climate-data.org/america-do-sul/brazil/minas-gerais>, Accessed 25 de Set 2018
- Edmunds M (1974) Defence in animals: a survey of anti-predator defences, Longman New York, p 357
- Francisco GS, Souza MM, Clemente MA, Brunismann AG (2018) Substrato vegetal utilizado para nidificação de vespas sociais (Hymenoptera, Vespidae) em Floresta Decidual. *Revista Agrogeoambiental* 10:35–45
- Giannotti E, Trevisoli C (1993) Desenvolvimento pós-embrionário de *Mischocyttarus drewseni* Saussure, 1857 (Hymenoptera, Vespidae) *Insecta* 2(2):41–52
- Giannotti E (1998) The colony cycle of the social wasp *Mischocyttarus cerberus styx* Richards, 1940 (Hymenoptera, Vespidae). *Rev Bras Entomol* 41(2–4):217–224
- Giannotti E (1999) Arquitetura de ninhos de *Mischocyttarus cerberus styx* Richards, 1940 (Hymenoptera, Vespidae). *Revista Brasileira de Zoociências* 1(1):7–18
- Giannotti E, Machado VLL (1999) Behavioral castes in the primitively eussocial wasp *Polistes lanio* Fabricius (Hymenoptera: Vespidae). *Rev Bras Entomol* 43:185–190
- Gobbi N, Fowler HG, Chaud-Netto J, Nazareth SL (1993) Comparative colony productivity of *Polistes simillimus* and *Polistes versicolor* (Hymenoptera: Vespidae) and the evolution of paragyny in the Polistinae. *Zoologische Jahrbucher Physiology* 97:239–243
- Gonzalez RC, Woods RE (2009) *Processamento Digital de Imagens* 3 ed. [S.l.]: Pearson
- Hermann HR, Blum MS (1981) Defensive mechanisms in the social Hymenoptera. In: Hermann HR (ed) *Social insects*. Academic Press, New York, pp 77–97
- Herre EA, Windsor DM, Foster RB (1986) Nesting associations of wasps and ants on lowland peruvian ant-plants. *Psyche* 93:321–330
- Jeanne RL (1975) The adaptiveness of social wasp nest architecture. *Q Rev Biol* 50:267–287
- Lawton JH (1983) Plant architecture and the diversity of phytophagous insects. *Ann Entomol Soc Am* 28:23–39
- Little M (1979) *Mischocyttarus flavitarsis* in Arizona: social and nesting biology of a polistine wasp. *Zeitschrift Fur Tierpsychologie* 50:282–312
- Milani LR, Prezoto F, Clemente MA, Gomes PP, Souza MM (2020) Nesting behaviour of a Neotropical social wasp *Mischocyttarus saussurei* Zikán, 1949 (Hymenoptera, Vespidae). *Sociobiology* 67(1):121–125. <https://doi.org/10.13102/sociobiology.v67i1.4842>
- Montagna TS, Torres VO, Fernandes WD, Antonialli-Junior WF (2010) Nest architecture, colony productivity, and duration of immature stages in a social wasp, *Mischocyttarus consimilis*. *J Insect Sci* 10:1–12. <https://doi.org/10.1673/031.010.19101>
- Montagna TS, Antonialli-Junior WF (2016) Morphological differences between reproductive and non-reproductive females in the social wasp *Mischocyttarus consimilis* Zikán (Hymenoptera: Vespidae). *Sociobiology* 63:693–698. <https://doi.org/10.13102/sociobiology.v63i1.854>
- Olberg RM, Worthington AH, Fox JL, Bessette CE, Loosemore MP (2005) Prey size selection and distance estimation in foraging adult dragonflies. *J Comp Physiol A* 191(9):791–797. <https://doi.org/10.1007/s00359-005-0002-8>
- Oliveira-Filho AT (2006) Definição e delimitação de domínios e subdomínios das paisagens naturais do estado de Minas Gerais. In: Scolforo JR, Carvalho LMT. *Mapeamento e Inventário da Flora e dos Reflorestamentos de Minas Gerais*. Lavras: UFLA, pp 1–35
- Oliveira SA, Castro MM, Prezoto F (2010) Foundation pattern, productivity and colony success of the paper wasp, *Polistes versicolor*. *J Insect Sci* 10:1–10. <https://doi.org/10.1673/031.010.12501>
- Penna MAH, Gobbi N, Giacomini HC (2007a) An evaluation of the productivity of *Mischocyttarus drewseni* in a semi-urban environment (Hymenoptera: Polistinae). *Sociobiology* 50:113–120
- Penna MAH, Gobbi N, Giacomini HC, Prezoto F, Gonçalves FMF (2007) Comparative productivity of *Mischocyttarus cerberus styx* (Richards, 1940) and *Mischocyttarus cassununga* Saussure (von Ihering, 1903) in an anthropic environment as evaluation for differences in ecological strategies. *Zoociências* 9(2):205–212
- Pickett KM, Carpenter JM (2010) Simultaneous analysis and the origins of sociality in the Vespidae (Insecta: Hymenoptera). *Arthropod Systematics* 68:3–33
- Pike TW (2018) Quantifying camouflage and conspicuousness using visual salience. *Methods Ecol Evol* 9:1883–1895

- R CORE TEAM R: (2017) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rafael JA, Melo GAR, Carvalho CJB, Casari SA, Constantino R (2012) Insetos do Brasil. Diversidade e Taxonomia: Holos Editora 810p
- Richards OW (1978) The social wasps of the Americas, excluding the Vespinae. British Museum (Natural History), London, p 580
- Sage D (2018) Color segmentation: ImageJ plugin to cluster color pixel driven by the user input, Biomedical Imaging Group, EPFL, Available to: <http://bigwww.epfl.ch/sage/soft/colorsegmentation/>
- Santos GMM, Gobbi J, Cruz JD, Marques OM, Gobbi N (2009) Diversidade de vespas sociais (Hymenoptera: Vespidae) em áreas de cerrado na Bahia. Neotropical Entomology 38:317–320. <https://doi.org/10.1590/S1519-566X2009000300003>
- Schneider C, Rasband W, Eliceiri KNIH (2012) Image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675
- Scobie AA, Starr CK (2012) Nest structure of the neotropical social wasp *Mischocyttarus baconi* (Hymenoptera: Vespidae). Sociobiology 59(1):235–239. <https://doi.org/10.13102/sociobiology.v59i1.680>
- Silveira OT (2008) Phylogeny of wasps of the genus *Mischocyttarus* de Saussure (Hymenoptera: Vespidae, Polistinae). Rev Bras Entomol 54:510–549
- Silveira OT, Silva SS, Felizardo SPS (2015) Notes on social wasps of the group of *Mischocyttarus (Omega) punctatus* (Ducke), with description of six new species (Hymenoptera, Vespidae, Polistinae). Rev Bras Entomol 59:154–168
- Smith EF (2004) Nest sites of the paper wasp *Mischocyttarus collarellus* (Hymenoptera: Vespidae: Polistinae) in a lowland tropical rain forest. J Kansas Entomol Soc 77(4):457–469
- Somavilla A, Oliveira ML, Silveira OT (2012) Guia de identificação de ninhos de vespas sociais (Hymenoptera, Vespidae, Polistinae) na Reserva Ducke, Manaus, Amazonas, Brasil. Rev Bras Entomol 56:405–414
- Souza MM, Ladeira TE, Assis NRG, Elpino-Campos A, Carvalho P, Louzada JNC (2010a) Ecologia de vespas sociais (Hymenoptera, Vespidae) no Campo Rupestre na Área de Proteção Ambiental, APA, São José, Tiradentes. MG MG Biota 3:01–30
- Souza MM, Louzada JNC, Serrão EJ, Zanuncio JC (2010b) Social wasp (Hymenoptera, Vespidae) as indicators of conservation degree of riparian forest in southeast Brazil. Sociobiology 56(2):387–396
- Souza MM, Zanuncio, JC (2012) Marimbondos: vespas sociais (Hymenoptera: Vespidae). Editora UFV Viçosa p79
- Souza MM, Pires EP, Elpino-Campos A, Louzada JNC (2014) Nesting of social wasps (Hymenoptera: Vespidae) in a riparian forest of Rio das Mortes in southeastern Brazil. Acta Sci Biol Sci 36(2):189–196. <https://doi.org/10.4025/actasciobiolsci.v36i2.21460>
- Souza MM, Teofilo-Guedes GS, Milani LR, Souza ASB, Gomes PP (2020) Social wasp (Vespidae: Polistinae) from the Brazilian Atlantic Forest. Sociobiology 67(1):1–12. <https://doi.org/10.13102/sociobiology.v67i1>
- Souza MM, Clemente MA, Teofilo-Guedes GS (2020) Nest camouflage records on five social wasp species (Vespidae, Polistinae) from southeastern Brazil. EntomoBrasilis 13:e929. <https://doi.org/10.12741/ebrasilis.v13.e929>
- Starr CK (1991) The nest as the locus of social life. In: Ross KG, Matthews RW (eds) The social biology of wasps. Cornell University Press, Ithaca, pp 520–539
- Starr CK (2014) Things we don't know about West Indian social wasps. Living World, Journal of The Trinidad and Tobago Field Naturalists' Club, pp 74–81
- Strassmann JE, Hughes CR, Queller DC (1990) Colony defense in the social wasps, *Parachartergus colobopterus*. Biotropica, 22: <https://doi.org/10.2307/2388546>
- Torres OV, Montagna TS, Fernandes WD, Antonialli-Junior WF (2011) Colony cycle of the social wasp *Mischocyttarus consimilis* Zikán (Hymenoptera, Vespidae). Rev Bras Entomol 55(2):247–252. <https://doi.org/10.1590/S0085-56262011000200016>
- Turillazzi, S. (2012) The biology of hover wasps. Springer Heidelberg New York Dordrecht London, p 283
- Wenzel JWA (1998) Generic key to the nests of hornets, yellowjackets, and paper wasps worldwide (Vespidae: Vespinae, Polistinae). Am Mus Novit 3224:1–39

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.