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METHODOLOGY FOR EVALUATION THE DEFENSIVE BEHAVIOR OF COLONIES OF SOCIAL WASPS (VESPIDAE: POLISTINAE) IN RESPONSE TO EXTERNAL STIMULI[1]

Maria Luiza Simões Silva[2]
Fernando Gonçalves de Aguiar Crispim
Diego Gonçalves dos Santos Renne
Eike Daniel Fôlha Ferreira
Tomás Matheus Dias de Oliveira
Gabriel de Castro Jacques
Marcos Magalhães de Souza

ABSTRACT

Studies on the defensive behavior of social wasps are important for different reasons, such as to establish the management of these insects in agricultural areas, avoiding the destruction of colonies for fear of their supposed aggressiveness. Thus, this work aims to propose an additional methodology for evaluating the defensive behavior of social wasps, aiming mainly to estimate the frequency of sting use. The study was conducted with the social wasp species *Parachartergus pseudapicalis* Willink, through the *ad libitum* method at the Fazenda Escola of IFSULDEMINAS, *campus* Inconfidentes, Minas Gerais, Brazil, from March to June, 2022, with 26 hours of ethological records. The methodology determines the use of visual, sound, and mechanical stimuli at different distances from the colony and in a given time frame, aiming at observing their possible reactions to generate statistical results. Thus, the methodology presented is relevant because it proved to be fast, practical, low cost and low impact on biodiversity, which is an important step to develop more studies using it. But to obtain more accurate results, it is necessary to replicate the method in other research.

Keywords: Sampling. Conservation. Ethology. Epiponini. *Parachartergus*.

METODOLOGIA PARA AVALIAÇÃO DO COMPORTAMENTO DEFENSIVO DAS COLÔNIAS DE VESPAS SOCIAIS (VESPIDAE: POLISTINAE) EM RESPOSTA A ESTÍMULOS EXTERNOS

RESUMO

Estudos sobre o comportamento defensivo das vespas sociais são importantes por diferentes razões, como por exemplo estabelecer o manejo destes insetos em áreas agrícolas, evitando a

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[2]**Autor correspondente:** Maria Luiza Simões Silva, e-mail: maria19.silva@alunos.ifsuldeminas.edu.br.

destruição das colônias por temor de sua agressividade. Assim, o objetivo do presente trabalho é propor uma metodologia adicional para avaliar o comportamento defensivo das vespas sociais, com o objetivo principal de estimar a frequência da ferroada. O estudo foi realizado com a espécie de vespa social *Parachartergus pseudapicalis* Willink, através do método *ad libitum* na Fazenda Escola do IFSULDEMINAS, *campus* Inconfidentes, Minas Gerais, Brasil, de março a junho de 2022, com 26 horas de registros etológicos. A metodologia determina o uso de estímulos visuais, sonoros e mecânicos a diferentes distâncias da colônia em um tempo determinado, com o objetivo de observar suas possíveis reações para gerar resultados estatísticos. Assim, a metodologia apresentada é relevante porque demonstra ser rápida, prática, de baixo custo e baixo impacto na biodiversidade, a qual é um passo importante para desenvolver mais estudos usando-a. Contudo, para obter resultados mais precisos é necessário replicar o método em outras investigações.

Palavras-chave: Amostragem. Conservação. Etologia. Epiponini. *Parachartergus*.

1 INTRODUCTION

Social wasps (Vespidae: Polistinae) present different behaviors and mechanisms for colony protection, such as camouflage (MILANI et al., 2021; SILVA et al., 2022), chemical defense (JEANNE, 1970), nest architecture (RICHARDS; RICHARDS, 1951; ALVARENGA et al., 2010), and aggressiveness (WENZEL, 1998), which is characterized by the ability of females to sting, through their ovipositor modified into stinger (RICHARDS, 1971), behavioral defense mechanism based on display (HERMANN; KELTING; CAPOBIANCO, 2023). These insects perform different ecosystem services, such as pollination (BRODMANN et al., 2008; MELLO et al., 2011) and predation of different animal species (FRANKHUIZEN; LOPES; CUNHA, 2020; OLIVEIRA; RUBIM; SOUZA, 2023), including agricultural pests, therefore, are used in biological control (ELISEI et al., 2010; PREZOTO et al., 2019; JACQUES et al., 2019; PINHEIRO et al., 2023). However, ignorance of this ecological role and fear of stings, based on supposed aggressiveness, explains the destruction of their nests by people (NORONHA et al., 2021).

Studies on the defense behavior of social wasps are relevant as they can help in the management of their colonies in agricultural areas, in order to reduce accidents with stings and avoid the destruction and removal of nests, which contributes to the conservation of local biodiversity, in addition ensuring the environmental services provided by these insects (PREZOTO, 1999). Some studies have already proposed or used methodologies to measure colony defense behavior (CHAVARRÍA-PIZZARRO, WEST-EBERHARD 2010; BRITO;

ARAGÃO; SANTOS, 2018; SILVA et al., 2022; SOUZA et al., 2022). For instance, Hermann, Kelting and Capobianco (2023) presents a methodology to evaluate the distinct defense behaviors of social wasps, filming the colony with the aid of a câmera, when it is under mechanical stress.

The aim of this study is to propose an additional methodology to evaluate the defensive behavior of social wasp colonies, in particular the sting behavior, in response to different external stimuli: (i) mechanical shock, such as Hermann, Kelting and Capobianco (2023); (ii) sound and (iii) visual disturbance. In addition, we aimed to testing a safe distance to avoid sting accidents, with the function of being a faster, more practical test, with less costs and, above all, with less impact on biodiversity, since it is not necessary to remove the colony from the natural environment.

2 MATERIALS AND METHODS

2.1 Place and period of study

This study was carried out at the Fazenda Escola of Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais (IFSULDEMINAS), c*ampus* Inconfidentes, Minas Gerais (22°18'32.7” S 46°19'46.1” W), in an anthropized area. To test the additional methodology for measuring the aggressiveness of social wasps, a colony of *Parachartergus pseudapicalis* Willink, 1959, nesting at 2.5 meters above the ground, was chosen. Observations in the colony occurred during autumn and early winter, from March 21 to June 27, 2022, with 26 hours of ethological records. The choice of this colony was due to the ease of observation, as it is considered an aggressive species (BRITO; ARAGÃO; SANTOS, 2018; MARQUES, 1996) and occurs in agricultural areas in the region of the study proposed here (FERREIRA et al., 2022).

2.2 Evaluation of the defense behavior of social wasps

We used the *ad libitum* method (DEL-CLARO, 2010), which consists of observations with standardized time intervals for recording important activities. For behavioral recording, we registered the occurrences in observation sessions lasting approximately 1.5 hours. The sessions were divided into three stages; the distances of the stimuli emission by the researcher were four meters, two meters, and below the nest, each with a duration of 15 minutes and an interval of 10 minutes between them, aiming at not overlapping the stimuli. In each stage, five stimuli were performed with a duration of 1 minute each. Two researchers made the observations: one promoted the stimuli, wearing beekeeper's clothing for protection and the other noted the reactions of social wasps.

The stimuli were performed in sequence: 1 - "visual" (Fig. 1A), the researcher remains silent and without movements; 2 **- "**movement" (Fig. 1B), which consisted of the movement of the researcher's arms up and down; 3 - "noise" (Fig. 1C), emission of three screams by the researcher; 4 "noise and movement" (Fig. 1D); and 5 **-** "touch" (Fig. 1E), which consists of directly touching the support branch in the nest, from below the nest. The choice of stimulus type was based on studies that show that social wasps present defensive behavior when they suffer visual (JEANNE, 1982), mechanical (DONNEL; JEANNE, 1992), and noise disturbance (BRUSCHINI; CERVO; TURILLAZZI, 2005) (Fig. 1).

The experiment was carried out over consecutive weeks or fortnightly to reduce the overlap of stimuli, as Brito, Aragão and Santos (2018) suggested, avoiding excessive stress, which could negatively interfere in the analyses. The observations were made three times a week on alternate days, with different times for each observation day (9h-10h30; 12h-13h30; 15h-16h30) in order to verify the possible behavioral changes in response to temperature, which were made available according to times and dates by Dr. Fernando da Silva Barbosa, according to the weather station installed on the farm school of IFSULDEMINAS, *campus* Inconfidentes.

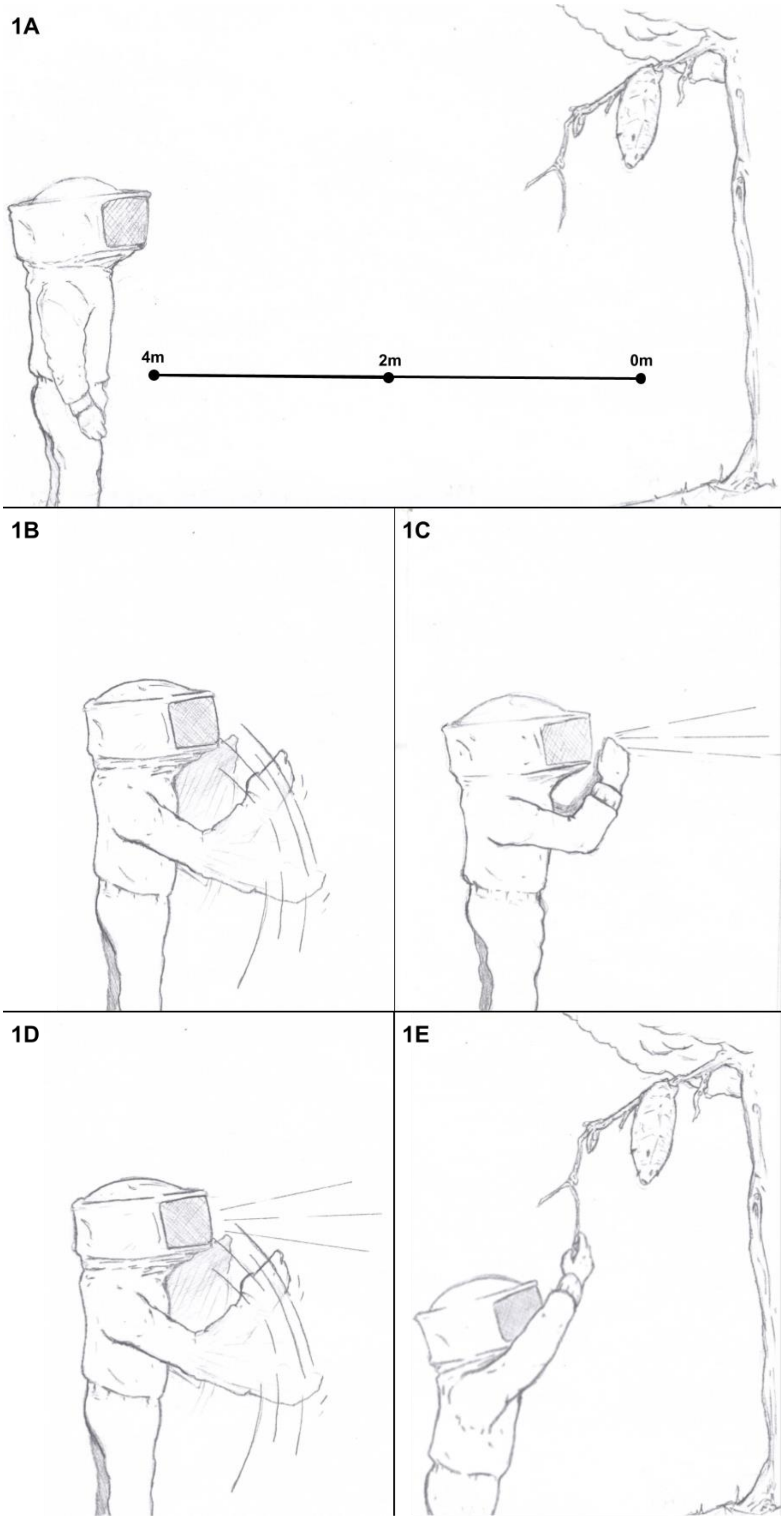

Figure 1. Stimuli performed by the researcher. **A)** ''Visual'' stimulus, **B)** ''Movement''stimulus, **C)** ''Noise'' stimulus, **D)** ''Noise and movement'' stimulus, **E)** ''Touch'' stimulus. **Source:** The authors.

2.3 Statistical Analysis

The data of presence or absence of reactions of social wasps to each stimulus and distance were tabulated, with scores of 1 or 0 in all sections, where 0 corresponds to no reaction of the wasps to the stimuli, and 1 to some reaction, regardless of what it was at that moment. Initially, the Shapiro-Wilk hypothesis test was performed to check the data's normality. Then, with these obtained data, the Kruskal-Wallis H test (KW) was conducted to verify if there was a significant difference in the number of presence or absence of reactions of social wasps at different distances (below the nest, zero distance, and two and four meters) and to different stimuli (visual, movement, noise, noise and movement, and touch). In case of statistical difference, the Mann-Whitney U test was performed in pairs, using the Past 4.03 program (Hammer; Harper; Ryan, 2001). Thus, from the percentage of reaction presence (1) out of the total in which each stimulus was applied and its respective distance, it was possible to establish the degree of reaction of social wasps to these stimuli, considering low reaction (less than 25%); medium reaction (25% to 50%); and high reaction (above 50%).We selected the stimuli and their distances that obtained a result greater than 25% to presence of reaction (1) to analyze the types of reactions of each social wasp, classifying them with 1 (presence of the given reaction) and 0 (absence of the given reaction). Such reactions were categorized into: A) Attack: wasp sting in the researcher or poison spray; B) Display: consecutive contractions of the abdomen and lifting of the wings; and C) Perimeter: flight around the researcher or nest. This analysis was also performed through the Kruskal-Wallis H test and the Mann-Whitney U test, pair-by-par, through the Past 4.03 program (HAMMER; HARPER; RYAN, 2001). The number of wasps attacks was analyzed at different temperatures (15°C to 20°; 21 to 25°C; and 26°C to 30°C) through the Kruskal-Wallis H test and then, the Mann-Whitney U test, pair-by-pair, through the Past 4.03 program (HAMMER; HARPER; RYAN, 2001).

2.4 Collection and Identification

Some specimens from the colony were captured with an entomological net and preserved in a container with 70% alcohol to identify the species. Dr. Marcos Magalhães de Souza performed the identification through identification keys (RICHARDS, 1978), with aid from the doctorate student Marcos Aragão from the Instituto Nacional de Pesquisa da Amazônia (INPA) and assistance from the Coleção Brasileira de Vespas Sociais (CBVS) of IFSULDEMINAS, *campus* Inconfidentes, where the wasps were incorporated into the collection (register number 08126-2019 and 08127-2019).

3 RESULTS AND DISCUSSION

3.1 Individual's reactions to stimuli and temperature

We obtained low (less than 25%) reactions to all stimuli from a distance of four meters and "visual" stimulus at all distances from the colony, in addition to "movement" at two meters; medium (25 to 50%) reactions to stimuli "noise" and "noise and movement," both two meters from the nest, and "movement" below the nest; and high (above 50%) response to the stimuli "touch," "noise and movement," and "noise," all below the nest (Tab. 1).

Table 1. Mean reactions and types of responses (attack, display, and perimeter) of *Parachartergus pseudapicalis* from different stimuli (touch, noise and movement, noise, movement, visual) and distances (zero, two, and four meters). The types of reactions were only estimated for stimuli that caused more than 25% of reactions (Means with different capital letters in the "Reactions" column differ by the Mann-Whitney U Test at 5% significance; Means with different lowercase letters in the column of each type of reaction differ by the Mann-Whitney U Test at 5% significance).

Distance	Stimulus	Reactions	Type of reaction		
			Attack	Display	Perimeter
0m					
	Touch	0.97A	0.46a	0.53a	0.62a
	Noise and movement	0.77B	0.36ab	0.46ab	0.44b
	Noise	0.55C	0.06e	0.53a	0.26c
	Movement	0.56C	0.18cd	0.37ab	0.26c

	Visual	0.18E	-	-	-
2 m	Noise and movement	0.56C	0.28bc	0.40ab	0.33b
	Noise	0.38D	0.08de	0.36b	0.22cd
	Movement	0.27DE	0.08de	0.13c	0.14d
	Visual	0.09EF	-	-	-
4m	Noise and movement	0.06F	-	-	-
	Noise	0.12EF	-	-	-
	Movement	0.04F	-	-	-
	Visual	0.09EF	-	-	-

Source: The authors.

The “display” reaction, which is the first reaction of the colony, in which wasps stand on the nest in a state of alert by a threat, occurs almost the same in all stimuli, indicating a state of attention of the colony.

The “perimeter” reaction, in which the wasps come out of the colony in a more explicit demonstration of nest protection, occurs more often in touch, followed by noise and movement together, regardless of whether it is below the nest or two meters.

The stimuli “touch” and “noise and movement” below the nest were the ones that caused a greater number of attacks. The stimulus with the second greatest chance of an attack is the “noise and movement” from two meters and "movement" below the nest. The other stimuli cause low attack reaction (<10%).

The temperature ranges from 26 to 30 °C presented the greatest number of attacks (stings and poison spray) (p=0.005). Among the temperature ranges of 15° to 20 °C and 21 °C to 25 °C, the percentage of attacks is similar (p=0.611). We recorded no attacks below 17 °C.

In our study, sting events occur more frequently in response to touching the branch that supports the nest, which resulted in the highest number of attack responses (44%). This likely happens because it represents a direct threat to the physical integrity of the colony, a condition that can be exacerbated if the colony is in a post-emergent phase, as shown in the work of Brito, Aragão and Santos (2018). Therefore, promoting contact with the substrate supporting the nest when the colony is in a post-emergent phase could increase the risk of a person being stung. However, although less frequent, there is a possibility of a stinging response from zero meters to two meters by the "noise and movement" stimulus, so the suggested safe zone around the colony would be from a distance of two meters onwards. The “noise and movement” was the second stimulus with the highest number of attacks, causing responses up to the distance of two meters from the nest, which agrees with the ideas of Bruschini; Cervo and Turillazzi (2005) and Jeanne (1982) that the wasps respond to noise and visual stimuli. Thus, the noise was probably the stimulus that encouraged the exit of individuals from the nest, whereas the movement caused an overlap of stimuli, accentuating stress in the colony. On the other hand, the separate noise stimulus causes little risk of stinging regardless of distance. This stimulus triggers the colony's first defense reaction, the “display”.

The wasps presented a higher number of attacks (stings and sprays) from 26 °C to 30 °C temperatures, which could be explained by their metabolism increase in this temperature range (KÄFER; KOVAC; STABENTHEINER, 2012) since they are ectothermic and require the exchange of heat with the environment for thermoregulation of their body (MAY, 1979). Otherwise, at temperatures below 17 °C, we recorded no attacks, which could be explained by the metabolism reduction that occurs below this temperature (RODRIGUES, 2004). Another response that relates the environmental temperature to the colony defense is seen in the studies by Klingner, Richter and Schmolz (2006), which showed that temperatures from 25 and 34 °C are favorable for the development of larvae, which proves advantageous for wasps since they can decrease the energy investment in the thermoregulation of the colony and invest in other activities, such as the colony defense, which was also observed by Canevazzi and Noll (2011) in *Polybia paulista* (Von Ihering, 1896).

Some studies have shown that the degree of aggressiveness is influenced more by the stage of development of the colony than by the size of the population (JUDD, 1998), in which the number of immature individuals in the nests is positively correlated with the levels of aggressiveness (SPRADBERY, 1973). In swarming social wasps, normally, the number of workers and immatures, as well as the size of the colony, increases throughout the colony cycle (LONDON; JEANNE, 2003). However, there are specific changes in the proportion of immatures at each stage of development in a colony. Annual colonies can produce a single annual brood and perennial colonies can produce subsequent broods in a year (JUDD, 1998). Therefore, it is not possible to determine the number of immatures, and consequently, whether that colony is in a more aggressive phase or not, without destroying the colony for this evaluation, which negatively affects the local populations of these insects.

Based on this information, the methodology proposed here is relevant for studies that aim at evaluating the defense responses of social wasps, especially the sting, based on external stimuli. Our methodology also adds conservation value, since it is not necessary to destroy the colony in the process. In addition, the methodology is carried out quickly, as it can assess the colony's behavior in around 90 minutes; and only beekeeper equipment is required, which adds economic value to the method.

Some studies have already proposed or used methodologies to measure colony defense behavior, for example, Brito, Aragão, and Santos (2018) used a capture device 15 cm from the entrance to the nest, from which they collected the social wasps that left after a visual and vibrational disturbance; Souza et al., (2022) directly manipulated the nest, removing part of the protective envelope, and analyzed the behavior of these wasps, a similar procedure adopted by Chavarría-Pizzarro and West-Eberhard (2010); while in other studies, he evaluated the behavioral response by approaching the colony to capture individuals, which caused mechanical stress promoted by the entomological net in the nest (SILVA et al., 2022).. The study by Hermann, Kelting and Capobianco (2023) also discusses the defensive responses of social wasps of the genus *Polistes* spp. that build nests without the protective envelope in response to mechanical stimuli. Our study adds information to the work of Hermann, Kelting and Capobianco (2023) regarding the distance from the nest at which stinging is possible because it deals with different stimuli, such as visual and auditory stimuli.

4 CONCLUSION

The methodology proposed here for assessing the use of stinging as a defense mechanism in social wasp colonies in response to different external stimuli adds valuable information to existing research. It has proven to be a quick, practical, low-cost, and low-impact method on biodiversity, which is an important step towards conducting more studies using it. Additionally, it creates the possibility of managing these social insects in agricultural ecosystems by establishing a safe distance from the colony. However, to obtain a more significant result, it is necessary to replicate this methodology in a larger number of colonies during a prolonged period and using different species.

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DADOS DOS AUTORES:

Maria Luiza Simões Silva

E-mail: simoesmarialuiza567@gmail.com
Curriculum Lattes: http://lattes.cnpq.br/2349020467943580
Graduanda do curso de Licenciatura em Ciências Biológicas pelo Instituto Federal do Sul de Minas (IFSULDEMINAS), campus Inconfidentes. Faço parte do grupo de estudos do Laboratório de Zoologia, onde também produzo trabalhos científicos na área de aracnologia, entomologia e ecologia como estagiária voluntária. Tenho experiência em campo graças a campanhas fornecidas pelo laboratório em diferentes Unidades de Conservação e outras locais, sobretudo nos biomas de Mata Atlântica e Cerrado. Além disso, tenho vivência com educação ambiental e participei do Programa Institucional de Bolsas de Iniciação à Docência (PIBID) e no Programa Institucional de Residência Pedagógica.

Fernando Gonçalves de Aguiar Crispim
E-mail: fernando.crispim@alunos.ifsuldeminas.edu.br
Curriculum Lattes: http://lattes.cnpq.br/5641601791944229
Graduando em Licenciatura em Ciências Biológicas do IFSULDEMINAS - Campus Inconfidentes, Estagiário no Laboratório de Zoologia do IFSULDEMINAS - Campus Inconfidentes.

Diego Gonçalves dos Santos Renne
E-mail: rennedgs@gmail.com
Curriculum Lattes: http://lattes.cnpq.br/4521654713110165
Possui graduação em Licenciatura em Ciências Biológicas pelo Instituto Federal do Sul de Minas campus Inconfidentes (2024), membro do grupo de estudos do Laboratório de Zoologia, cujo o principal foco é desenvolver trabalhos com ênfase na ecologia e etologia de vespas sociais (Hymenoptera, Vespidae), libélulas (Odonata) e opiliões (Arachnida, Opiniones). Atualmente, Mestrando do Programa de pós Graduação em Entomologia pela Escola Superior de Agronomia Luiz de Queiroz (ESALQ-USP) onde desenvolvo pesquisas principalmente com vespas sociais (Hymenoptera, Vespidae).

Eike Daniel Fôlha Ferreira
E-mail: eikedanieel98@gmail.com
Curriculum Lattes: http://lattes.cnpq.br/6943832077622636
Licenciado em Ciências Biológicas pelo Instituto Federal do Sul de Minas - Campus Inconfidentes (2024), com experiência em ecologia, etnozoologia e etologia, especialmente em Vespidae, Odonata e Opiliones. Desenvolveu e participou de diversas ações de extensão, atuando em educação ambiental, ecoturismo e inventários de fauna. Atualmente, é aluno de Mestrado no Programa de Pós-Graduação em Entomologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP-USP), com foco em taxonomia e ecologia da ordem Odonata.

Tomás Matheus Dias de Oliveira
E-mail: deoliveiratmd@gmail.com
Curriculum Lattes: http://lattes.cnpq.br/0913764777332324
Licenciado em Ciências Biológicas no Instituto Federal do Sul de Minas - Campus Inconfidentes, onde atuei na Educação Básica nos respectivos programas PIBID e Residência Pedagógica (CAPES), e pude realizar pesquisas com grupos de artrópodes no Laboratório de Zoologia. Atualmente sou aluno de Mestrado em Entomologia pela Faculdade de Filosofia,

Ciências e Letras de Ribeirão Preto da Universidade de São Paulo (FFCLRP-USP), sob orientação do prof. Dr. Jean Carlos Santos (UFS). Realizo trabalhos na área de Entomologia, sendo a minha principal área de estudo a ordem Odonata.

Gabriel de Castro Jacques
E-mail: gabriel.jacques@ifmg.edu.br
Curriculum Lattes: http://lattes.cnpq.br/3848049450080555
Professor efetivo do Instituto Federal de Minas Gerais - IFMG Campus Bambuí, onde também atuou como Diretor de Pesquisa, Inovação e Pós-graduação. Doutor em Entomologia pela Universidade Federal de Lavras. Mestre em Entomologia pela Universidade Federal de Viçosa, sendo que parte do mestrado realizou no Instituto Politécnico de Bragança - Portugal. Bacharel em Ciências Biológicas também pela Universidade Federal de Viçosa. Atua em projetos relacionados com controle biológico de pragas e levantamento da biodiversidade de insetos e aracnídeos.

Marcos Magalhães de Souza
E-mail: marcos.souza@ifsuldeminas.edu.br
Curriculum Lattes: http://lattes.cnpq.br/2334845279402555
Possui graduação em Ciências Biológicas pela Universidade Federal de Juiz de Fora (2000), mestrado em Comportamento animal pela Universidade Federal de Juiz de Fora (2005), doutorado em entomologia pela Universidade Federal de Lavras (2010). Pós-doutorado pela UFV (2011). Atualmente é professor do Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais, Campus Inconfidentes, coordenador do laboratório de zoologia, trabalha com artropodas, com ênfase em ecologia, etnozoologia e etologia de Vespidae, Odonata e Opiliones. São mais de 165 artigos. Desenvolve ações de extensão, atua em educação ambiental, ecoturismo e inventários de fauna.