



DIVERSITY OF PARASITOID WASPS (HYMENOPTERA) IN TWO FOREST FRAGMENTS: EVALUATING THE ROLE OF ENVIRONMENTAL VARIABLES

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Abstract. Understanding how environmental conditions shape insect diversity in human-modified landscapes is critical for biodiversity conservation in the Brazilian Cerrado. In this context, parasitoid hymenopterans may be effective biodiversity indicators because of their high trophic position and close ecological associations with host arthropods. In this study, we evaluated the abundance and richness of parasitoid wasp families in two Cerrado forest fragments in central Brazil differing in size and surrounding land use. Using yellow pan traps, we collected 247 specimens belonging to 19 families between August 2020 and February 2021. Aphelinidae, Bethylidae, Ichneumonidae, Mutillidae, Mymaridae, and Platygasteridae were the most abundant families. Generalized Linear Mixed Models indicated a negative effect of humidity on both richness and abundance, while area and temperature had no significant influence. No consistent temporal trends or differences between fragments were detected. These results suggest that regional environmental conditions, particularly humidity, play a key role in structuring parasitoid wasp assemblages in fragmented Cerrado landscapes and highlight the need for longer-term and multi-scale studies to better understand parasitoid community dynamics in tropical savannas.

Keywords: biodiversity indicators; Cerrado; community ecology; faunal survey; Mutillidae; Platygasteridae.

INTRODUCTION

Comprehending the mechanisms that maintain biodiversity is essential for a thorough understanding of ecosystem functioning; biodiversity constitutes a fundamental component in structuring species interactions and underpins the stability and resilience of ecological communities (Tilman et al. 2006, Yang et al. 2021).

The loss of biodiversity is particularly concerning in biomes such as the Brazilian Cerrado, recognized as a key area for biodiversity conservation due to its remarkable species richness and high levels of endemism (Mittermeier et al. 2011). The Cerrado has undergone intense deforestation due to expansion of agriculture and livestock, as well as infrastructure development, and the weak environmental regulation enforcement (Strassburg

et al. 2017, Colli et al. 2020, Guerra et al. 2020). Moreover, the Brazilian Cerrado is undergoing pronounced climate change, becoming increasingly hotter and drier, with a significant reduction in dewfall (Hofmann et al. 2021).

Insects are increasingly recognized as effective bioindicators due to their exceptional diversity, ecological specialization, and sensitivity to environmental changes (McGeoch 1998, 2007, Chowdhury et al. 2023). Their abundance, relatively short life cycles, and ease of sampling make them valuable tools for assessing ecosystem health and monitoring the impacts of environmental disturbances such as pollution, habitat loss, and climate change (McGeoch 2007, Chowdhury et al. 2023). Bioindicators reflect abiotic conditions, ecological responses to environmental stress, or even broader patterns of biodiversity (McGeoch 1998). Among insects, groups such as beetles, ants, butterflies, and gall-inducing insects have been widely used, the latter being particularly sensitive to habitat quality and disturbances (McGeoch 1998). Some of them, such as gall-inducing insects, are often associated with specific host plants and plant organs, and therefore tend to reflect local plant diversity and habitat conditions (Santana & Isaias 2014). Notably, parasitoid hymenopterans have gained attention for their potential as biodiversity indicators, given their high trophic position, intimate ecological associations with host arthropods, and responsiveness to habitat structure and landscape complexity (Anderson et al. 2011). Furthermore, the richness of parasitoid hymenopteran subfamilies has been shown to serve as a reliable indicator for species richness, offering a practical shortcut in biodiversity assessments under time or resource constraints (Mazón 2016). These characteristics demonstrate the potential of parasitoid hymenopterans, especially microhymenopteran groups, as an excellent tools for bioindication and biodiversity monitoring in natural and managed ecosystems.

Hymenoptera is one of the most diverse insect orders, occupying a wide range of ecological niches as parasitoids, phytophagous, pollinators, and even exhibiting eusocial behavior (Blaimer et al. 2023). Among them, parasitoid hymenopterans

are particularly megadiverse and may contribute to making this order the most species-rich group of animals globally (Forbes et al. 2018). These insects are characterized by a high degree of specialization and often occupy higher trophic levels, exerting control over other insect populations. For this reason, they are considered useful indicators of the conservation status of local arthropod communities (Shaw & Hochberg 2001, Anderson et al. 2011, Aranda & Graciolli 2015). Despite their ecological and economic importance in biological pest control, parasitoid wasps remain relatively understudied from a taxonomic perspective, largely due to their small size and inconspicuous behavior. As a result, microhymenopterans are often underrepresented in biodiversity surveys when compared to other insect orders (Heraty 2009, Haas-Renninger et al. 2024).

Environmental and habitat variables may influence parasitoid biodiversity, since they play a fundamental role in shaping the ecology, behavior, and population dynamics of parasitoid hymenopterans. Key abiotic factors such as temperature, humidity, and seasonality influence essential biological traits including mobility, foraging activity, survival and can significantly affect parasitism rates (Jerbi-Elayed et al. 2015, Wetherington et al. 2017). In parallel, seasonal fluctuations in resource availability contribute to temporal variations in parasitoid abundance. Additionally, habitat characteristics shaped by climatic gradients such as elevation and latitude further influence species richness and community composition (Kendall & Ward 2016, Schliserman et al. 2016, Flinte et al. 2023). Together, these findings underscore the complex and multifaceted impacts of both environmental and habitat factors on parasitoid wasps, with significant implications for their diversity in natural ecosystems.

Building on this framework, in this manuscript we evaluate the abundance and richness of parasitoid Hymenoptera in two distinct forest fragments within the Cerrado Domain, compare the diversity between these fragments, and assess the influence of environmental variables, specifically temperature and humidity, on the diversity of parasitoid wasps.

MATERIAL & METHODS

Studied area

The study was conducted in Rio Verde, Goiás state, Central Brazil (Figure 1A–C), located within the Cerrado Domain. The research spanned from August 2020 to February 2021 and included a total of 14 sampling events. The collections were temporally spaced by at least 15 days. Two areas were analyzed: forest fragments located at Instituto Federal Goiano – Rio Verde Campus: a smaller fragment (A1 with 2.3 hectares; lat. -17.805046° , lon. -50.903757°) and a larger fragment, (A2 with about 28 hectares; lat. -17.818931° , lon. -50.900248°) (Figure 1A–B). The fragments were approximately 1.5 km apart and situated in two distinct landscape

contexts: A1 was located near an urban area, while A2 was embedded within a more rural landscape. Using the *landscapemetrics* package (Hesselbarth et al. 2019), we extracted landscape metrics from *MapBiomas* (Souza Jr. et al. 2020) Collection 9 within a 0.5 km radius. Both areas displayed comparable percentages of agriculture and livestock; however, A1 landscape consisted of 7.4% natural areas, while A2 contained 38.3% at the observed scale (Figure 1C). Additionally, A1 exhibited 27.3% urban and non-vegetated areas, which were entirely absent in A2.

In each forest fragment 10 yellow Moericke-type traps (“pan traps”) were used, each with a diameter of 15 cm (Figure 1D–E). We employed a single trap type to standardize sampling method and ensure comparability among forest fragments and across multiple sampling events. Yellow pan traps, like

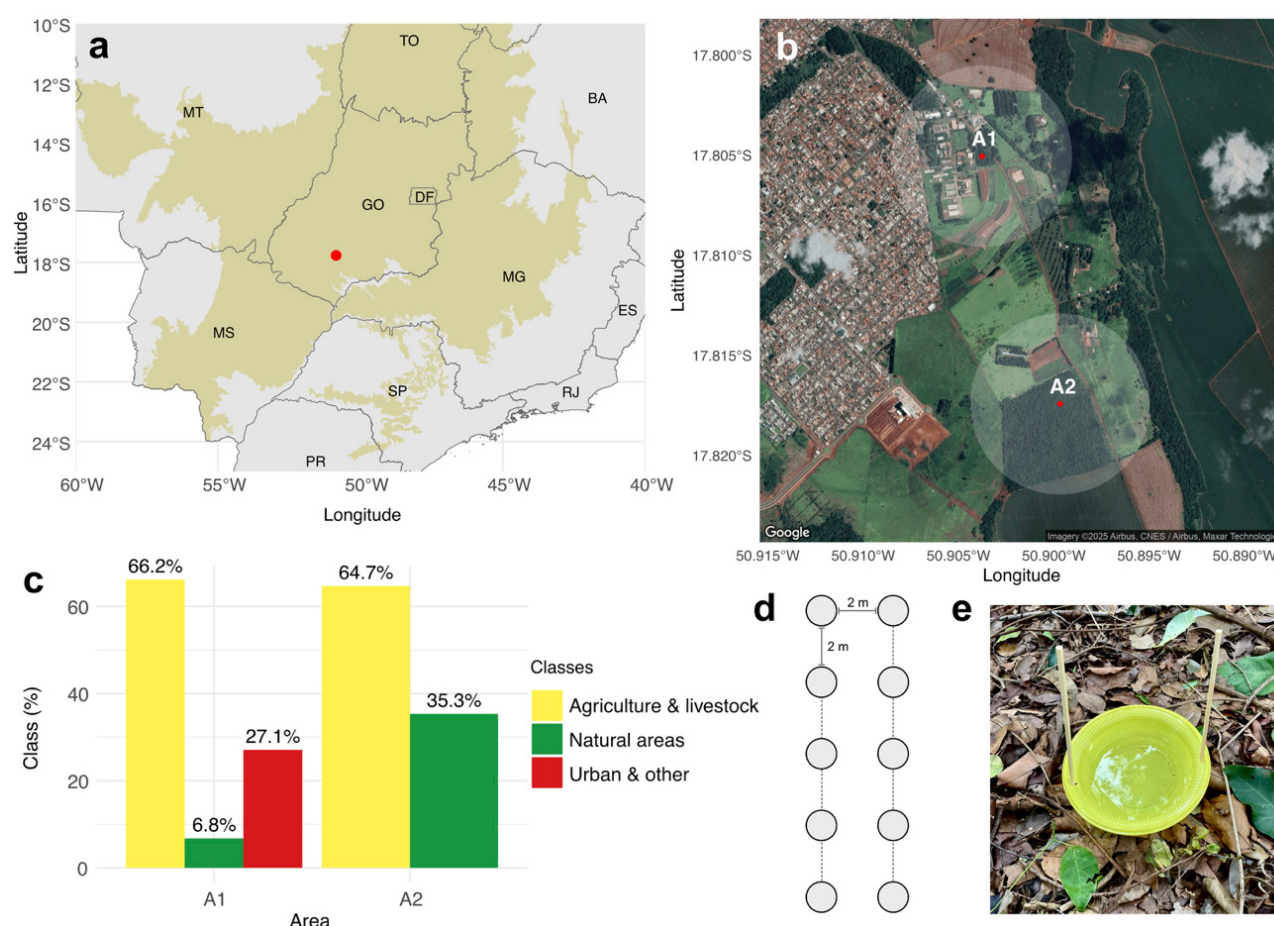


Figure 1. Sampling locality. **a**: Map of central Brazil with emphasis on Goiás state, showing the Cerrado Domain and the location of the study area (Rio Verde) indicated by a red dot; **b**: Satellite map depicting sampling units, with a 0.5 km buffer around fragments A1 and A2 (© Airbus, CNES/Airbus, Maxar Technologies); **c**: Bar plot showing the percentage distribution of landscape classes within a 0.5 km buffer around fragments A1 and A2; **d**: Sampling unit layout, where circles represent the placement of yellow pan traps; **e**: Image of a yellow pan trap used in this study.

those employed in this study, are known to often capture a greater diversity of insects (particularly Hymenoptera) compared to other colors, and are especially effective for sampling parasitic wasps (Buffington et al. 2020, Montgomery et al. 2021). The traps were arranged in two rows, with a spacing of 2 meters between them within the sampling area. The traps were placed at ground level and remained active for 24 hours period during each collection event. In each trap was filled with 100 mL of a solution of water and detergent (to break the surface tension of the water). Each sampling unit comprised the 10 traps deployed within a given area during each sampling interval. Three experimental replicates in A2 were lost due to environmental interference, potentially caused by animals, adverse weather conditions, or human disturbance. As a result, 14 samples were obtained in A1 and 11 in A2.

The water and detergent solution were filtered to separate the collected insects. Subsequently, the insects were preserved in 70% ethanol within Falcon tubes and stored under refrigeration.

Specimen Identification and environmental data collection

The specimens collected were and identified at the family level using identification keys (Goulet & Huber 1993, Gibson 1997, Fernández & Sharkey 2006, Melo et al. 2012). Given the high diversity and taxonomic knowledge gaps in the group, specimens were classified into recognizable taxonomic units within each sample for the estimation of sample-level richness. To ensure more effective preservation, the parasitoid wasps were stored in absolute ethanol. Voucher specimens are deposited at the Laboratório de Ecotoxicologia e Sistemática Animal at Instituto Federal Goiano – Rio Verde Campus.

Since parasitoidism occurs in multiple lineages of Hymenoptera, this study includes the following groups sensu Blaimer et al. (2023): (1) Ichneumonoidea, a diverse superfamily of parasitoid wasps; (2) Proctotrupomorpha, which encompasses several superfamilies of primarily parasitoid wasps; (3) various lineages within the “Evaniomorpha Grade,” including Stephanoidea, Evanioidea, Ceraphronoidea, Megalyroidea, and Trigonoidea. Additionally, we incorporated

aculeate Hymenoptera that exhibit a parasitoid lifestyle, specifically Chrysidoidea and Pompiloidea.

The abundance and richness of parasitoid Hymenoptera were calculated for each sample of 10 traps within each fragment. Daily average temperature and humidity data for the Rio Verde region were obtained from the National Institute of Meteorology (<https://portal.inmet.gov.br/>).

Data analysis

To assess whether the richness and abundance of parasitoid wasp families exhibited a temporal pattern, the autocorrelation function (ACF) was applied to both variables in each study area. Given the short time span and the limited number of sampling events, the analysis was conducted as an exploratory approach to detect potential temporal trends in the data. All analyses were performed using the *acf* function from the stats package in R v.4.5.0 (R Core Team 2025).

In our models, richness and abundance of parasitoid Hymenoptera were treated as response variables, and the effect of environmental variables including area (A1 and A2), humidity and temperature as fixed effects. Continuous predictor variables were scaled and centered prior to analyses. Since sampling within A1 and A2 were carried out concomitantly, we included sampling date as a random effect (1|sampling_date). Generalized Linear Mixed Models (GLMM) were fitted using the package *glmmTMB* (Brooks et al. 2017), assuming a Poisson distribution. For each response variable, a full model including all predictor variables was initially fitted. For each response variable, full models including all predictor variables were adjusted. Model simplification was then performed by sequentially removing predictors with low explanatory power to obtain the most parsimonious model. Nested models were then compared using likelihood ratio tests, and variables were retained in the final model only when their inclusion significantly improved model fit ($p < 0.05$). To assess overdispersion, tests based on two-sided simulation were applied using the *testDispersion* (type = “DHARMA”) function from the DHARMA package (Hartig 2024). The Model fit was evaluated by simulating residuals with DHARMA and analyzing QQ plots and residual vs. predicted value plots.

RESULTS

A total of 247 parasitoid hymenopteran specimens were collected: 162 individuals in the smaller forest fragment (A1 – 14 samples) and 85 in the larger forest fragment (A2 – 11 samples). The most abundant parasitoid Hymenoptera families (with 20 or more specimens) were Aphelinidae and Mymaridae (Chalcidoidea), Bethyridae (Chrysidoidea), Ichneumonidae (Ichneumonoidea), Platygastriidae (Platygastridae) and Mutillidae (Pompiloidea) (Figure 2A). The richness patterns of parasitoid Hymenoptera families per sample were overall similar to abundance patterns (Figure 2B). The median abundance per sample was 10.5 (quartiles:

6.25 – 14.2) at A1 and 7 (4.5 – 10.5) at A2 (Figure 2C), whilst the median richness was 8 (5.25 – 10.8) for A1 and 6 (4.5 – 9.5) for A2 (Figure 2D). However, no significant differences among fragments were observed for either abundance or richness (Table 1).

The autocorrelation analysis revealed that there was no significant temporal autocorrelation between the richness or abundance of parasitoid wasp families in the two studied areas (Figure 3). This indicates an absence of temporal structure in these variables over the period studied, of ~8-month of sampling.

After model selection, humidity was retained as a significant predictor in both richness and abundance models, exhibiting a negative relationship with the

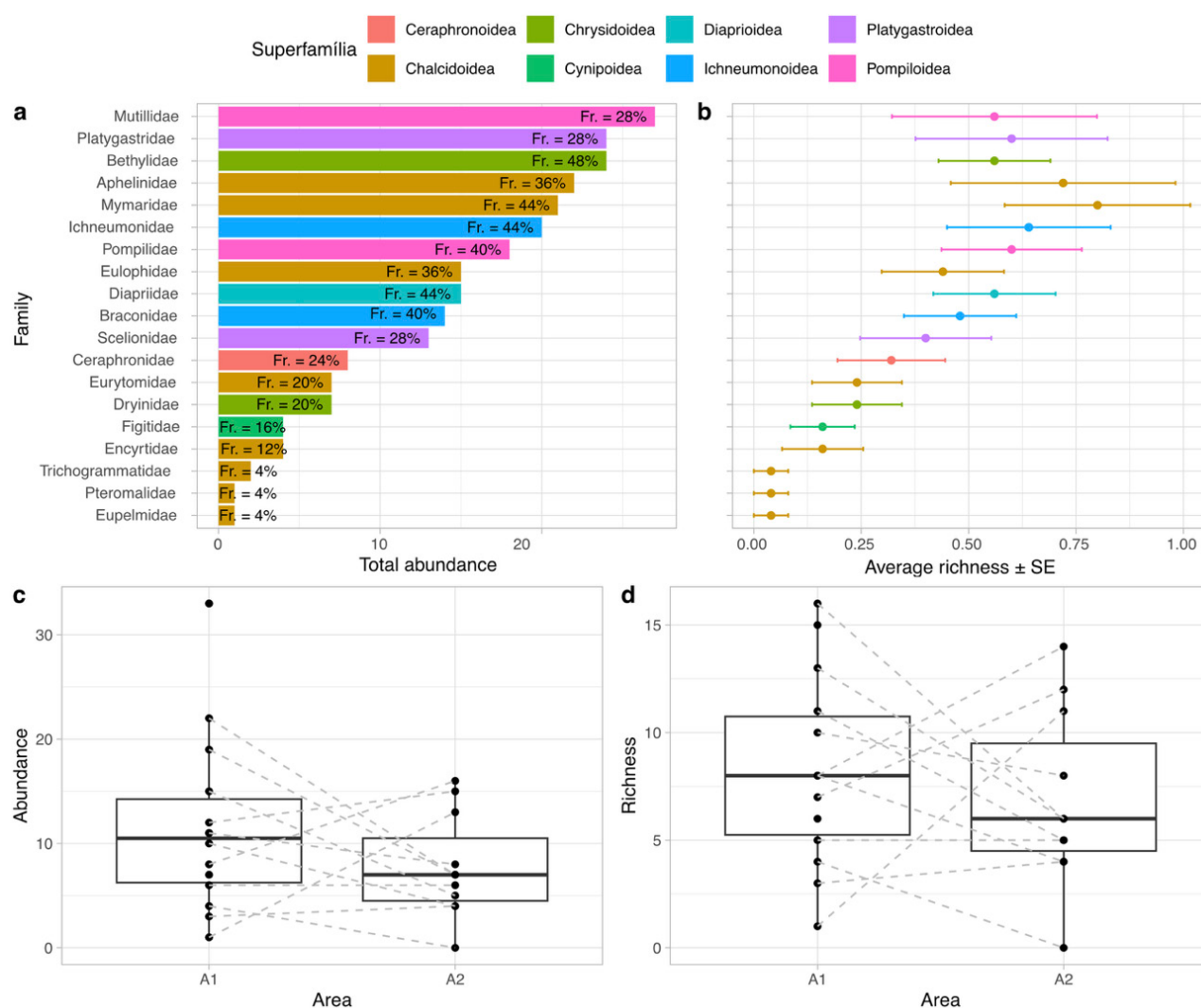


Figure 2. Richness and abundance of parasitoid Hymenoptera across the studied areas. **A:** Total abundance of sampled insects. Family frequency (Fr.) is indicated within the bars. **B:** Average richness per sample ($\pm$ standard error) for each family. **C & D** Boxplots comparing abundance (**C**) and richness (**D**) between the smaller forest fragment (A1) and the larger forest fragment (A2). Lines connect paired sampling points from the same date.

Table 1. Likelihood ratio tests comparing nested generalized linear mixed models for parasitoid abundance and richness. Models differed by the addition of individual predictors (area or temperature), while humidity was retained in all models. Log-likelihood values (logLik) are shown for each pair of compared models (a; b), where model a represents the more complex model. χ^2 and p-values correspond to likelihood ratio tests between nested models.

Response	Comparison a vs b	Dropped term	LogLik: a; b	χ^2	p-value
Abundance	ab1 vs ab2	temperature	-79.960; -79.965	0.01	0.922
	ab2 vs ab3	area	-79.965; -80.929	1.93	0.165
Richness	ri1 vs ri2	temperature	-66.644; -66.644	0.00	0.980
	ri2 vs ri3	area	-66.644; -66.668	0.05	0.829

Models:

ab1 & ri1: response ~ area + temperature + humidity + (1|sampling_date)

ab2 & ri2: response ~ area + humidity + (1|sampling_date)

ab3 & ri3: response ~ humidity + (1|sampling_date)

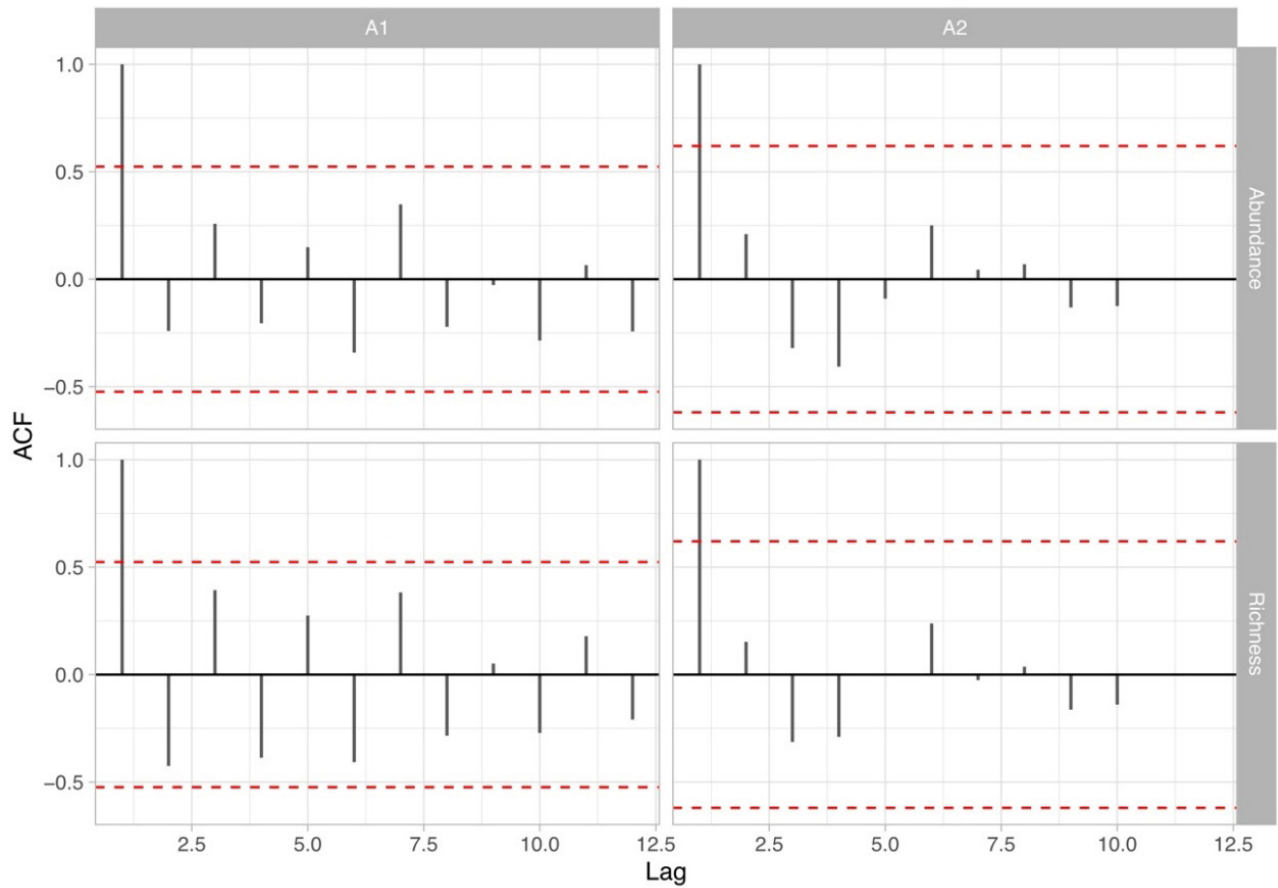


Figure 3. Autocorrelation function (ACF) plots for parasitoid wasp abundance and richness in areas A1 and A2.

response variables (Figure 4, Table 2). Temperature, in contrast, was not associated with richness or abundance of parasitoid wasp families (Figure 4; Table 1). The random effect of sampling date accounted for a small portion of the variance in

the richness model (ICC = 0.15), but represented a substantial component in the abundance model (ICC = 0.54; Table 2), indicating a stronger influence of sampling date on abundance patterns.

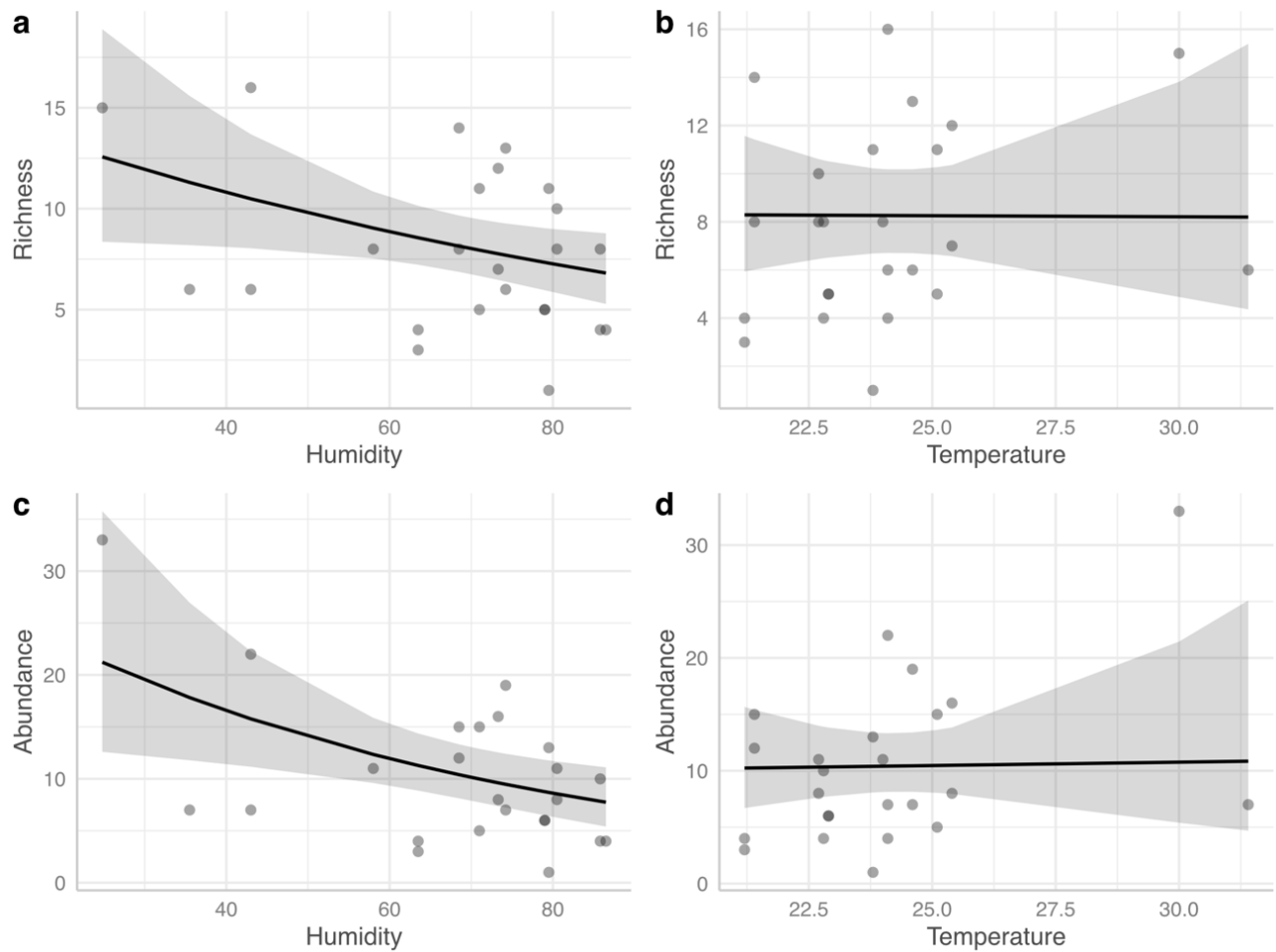


Figure 4. Scatterplots showing the relationships between richness (**a, b**) and abundance (**c, d**) with humidity (**a, c**) and temperature (**b, d**). The lines represent the fitted model, and the shaded areas indicate the 95% confidence intervals.

Table 2. Summary of the Generalized Linear Mixed Models (GLMM), presenting model parameters for the adjusted models, including both fixed and random effects. Significant p-values are shown in bold; IRR = Incidence Rate Ratios.

	Richness			Abundance		
Predictors	IRR	CI	p	IRR	CI	p
Intercept	7.70	6.49 – 9.13	<0.001	9.27	7.42 – 11.59	<0.001
Humidity (scaled)	0.85	0.73 – 0.99	0.032	0.75	0.62 – 0.90	0.003
Random Effects						
σ^2		0.12			0.09	
τ_{00}		0.02			0.10	
ICC		0.15			0.54	
Marginal R^2 / Conditional R^2		0.161 / 0.289			0.303 / 0.678	

DISCUSSION

In this study, richness and abundance of parasitoid hymenopteran assemblages were primarily related to environmental conditions rather than fragment size or short-term temporal variation. We observed broadly similar patterns of richness and abundance between the smaller and larger fragments, suggesting that, at the spatial scale considered, fragment sizes and landscape context did not affect richness and abundance parasitoid wasp families. Chalcidoidea, particularly Aphelinidae and Mymaridae, together with Ichneumonoidea, Platygastroidea, Bethylidae and Mutillidae, accounted for most of the sampled individuals, reflecting patterns commonly reported for parasitoid communities sampled with pan traps in Cerrado environments. Temporal autocorrelation analyses indicated no consistent seasonal structure in richness or abundance over the eight-month sampling period. In contrast, humidity was significant associated with richness and abundance, showing a negative relationship with both response variables, while temperature had no detectable effect. These findings highlight the predominance of local abiotic conditions, especially humidity, over fragment size and short-term temporal dynamics in shaping parasitoid wasp communities at a local scale in Cerrado forest remnants.

Parasitoid hymenopterans are a species-rich and abundant group of insects commonly found in both natural and anthropized ecosystems. Several studies have been carried out in natural habitats, particularly in forest remnants (Azevedo et al. 2002, Perioto & Lara 2003, Amaral et al. 2005), as well as in agroecosystems, including coffee, soybean, and horticultural crops (Perioto et al. 2002a, Souza et al. 2006, Ferreira et al. 2013). Others have examined interface environments or landscape mosaics, where native vegetation coexists with agricultural land use (Lara et al. 2015, Souza 2015). In contrast to these approaches, the present study examined two Cerrado forest fragments embedded in different landscape contexts at a local spatial scale. This framework allows an assessment of how local environmental conditions and surrounding landscape structure jointly influence parasitoid hymenopterans diversity in forest remnants.

In our study, we observed high richness and abundance of Mutillidae, Pompilidae, and

Bethylidae. These families were also recorded at low abundances in a study that examined interfaces between agricultural areas and natural Cerrado fragments (Lara et al. 2015), although Malaise traps were reported to be more effective for capturing these groups. Similarly, Ichneumonoidea exhibited a higher abundances in our samples than in those of Lara et al. (2015) using Moericke traps. However, our results were similar to those obtained with Malaise traps in their study, which similarly showed high abundances of Braconidae and Ichneumonidae. Ichneumonoids are commonly cited in parasitoid diversity surveys and are often among the most abundant and diverse groups in both natural and agricultural environments (Perioto & Lara 2003, Lara et al. 2015, Souza 2015), with particularly high abundances reported in Cerrado habitats (Moraes et al. 2012). Platygastroids were also among the most abundant groups in our samples, a pattern widely reported across extra-Amazonian Brazil (Perioto et al. 2002a, Perioto & Lara 2003, Souza et al. 2006, Moraes et al. 2012, Ferreira et al. 2013, Souza 2015), especially in studies that employed pan traps (Perioto & Lara 2003, Souza et al. 2006, Lara et al. 2015). At the superfamily level, Chalcidoidea was the most abundant group, with notable representation of the minute egg parasitoids Aphelinidae and Mymaridae. Mymarids are frequently among the most abundant families when Moericke traps are used (Marchiori & Penteado-Dias 2002, Souza et al. 2006), and they are also frequently reported in surveys conducted within Cerrado habitats (Perioto 1991, Marchiori & Penteado-Dias 2002, Lara et al. 2015). In contrast, Aphelinidae are typically not as abundant in natural environments (Marchiori & Penteado-Dias 2002, Moraes et al. 2012) with higher representation generally observed in agroecosystem studies (Perioto et al. 2002b, 2002a, Lara et al. 2015). Additionally, Encyrtidae, was the dominant family in the Cerrado studied by Lara et al. (2015), but showed low frequency in our samples. These patterns suggest that habitat structure, landscape context, and collection method influence the taxonomic composition, abundance and dominance of parasitoid wasp assemblages.

We found no significant differences in parasitoid Hymenoptera richness and abundance between forest fragments. In general, forest cover is often cited as an important factor for Hymenoptera diversity (Kendall & Ward 2016, Montagnana

et al. 2021); however, other factors such as habitat heterogeneity may also promote higher diversity (Montagnana et al. 2021), and structural complexity has been linked to functional diversity in these communities (Kendall & Ward 2016). Moreover, changes in land-use not only reduce species richness, but can also alter community composition, often favoring more generalist species (Simplicio et al. 2022). An important consideration in this context is spatial scale. Our comparison was made at a small scale, using a 500-meter radius around spatially close fragments. Given that microhymenopterans exhibit high dispersal ability (Compton et al. 2000, Esch et al. 2005, Evans 2018), it is possible that the spatial scale used may have been too narrow to fully capture the effects of habitat structure on parasitoid diversity. This could potentially allow both fragments to function as part of a shared regional species pool, as predicted from the metacommunity perspective (Leibold et al. 2004, Leibold & Chase 2018). Additionally, as this is a case study involving only two fragments of Cerrado during a limited period of time, it was not possible to disentangle the effects of other variables that may influence the landscape structure or microhabitat conditions.

Although seasonal climatic conditions are known to influence insect abundance, this effect may be less pronounced in tropical regions, where many species remain active for longer periods throughout the year. Nevertheless, even in these seemingly stable environments, insects often exhibit well-defined seasonal patterns (Wolda 1988). In the present study, sampling was conducted during the dry-to-rainy season transition. No clear temporal trend in parasitoid richness or abundance was detected; however, due to limited sampling duration (eight months) and the small number of traps deployed, these findings should be interpreted with caution. Temperature and humidity are generally critical factors influencing insect physiology and community dynamics (Singh et al. 2009), and also influence the life history of parasitic wasps (Gauld 1987). Higher humidity environments have been associated with greater insect diversity (Janzen & Schoener 1968). Interestingly, our results revealed a negative relationship between humidity and both parasitoid richness and abundance of Hymenoptera parasitoid families. This pattern aligns with other studies on

these groups, which have reported similar trends or abundance peaks during drier periods of the year (Aranda & Graciolli 2015, Santos et al. 2021). Such a pattern may reflect interacting ecological, behavioral, and physiological mechanisms. Environmental conditions such as humidity can indirectly influence parasitoid performance by mediating interactions with other natural enemies, particularly pathogens that compete for the same hosts, thereby altering host availability and competitive outcomes (Hochberg & Lawton 1990). In addition, many parasitoids exhibit higher activity in drier, warmer conditions (Weseloh 1979) and, in some cases, higher parasitism rates in drier substrates (Smith & Rutz 1991), while reproductive performance may peak under intermediate humidity levels (Kalyebi et al. 2006). Taken together, these patterns suggest that reduced movement under more humid or rainy conditions likely contributed to the lower capture rates observed.

This study evaluated a broad range of parasitoid Hymenoptera families, encompassing taxa with different levels of taxonomic knowledge and diagnostic complexity. This broad taxonomic scope, combined with the lack of comprehensive taxonomic reviews, makes it difficult to accurately identify most groups within the Hymenoptera, particularly for microhymenopterans. Here, species richness within parasitoid hymenopteran families was estimated using recognizable taxonomic units (RTUs) to ensure consistency across samples and fragments. Although the use of RTUs (or morphospecies) entails recognized limitations, especially for parasitoid Hymenoptera, where splitting and lumping errors may occur (Derraik et al. 2010), previous studies have demonstrated that such approaches can accurately capture patterns of relative species richness and community turnover, including in more complex analyses such as β -diversity (Oliver & Beattie 1996). Importantly, some limitations stem from biological realities, not methodological flaws, a key example is the widespread occurrence of cryptic species complexes in invertebrates (Vivien et al. 2025). Therefore, RTUs may provide informative operational units for comparative ecological analysis, given the current limitations in taxonomic resolution.

This study contributes to a better understanding of how local context and environmental structure shape parasitoid wasp communities in Cerrado

ecosystems. Several patterns observed here are consistent with broader trends reported in the literature, including the high species richness and abundance of Chalcidoidea and the influence of humidity on parasitoid assemblages. However, the limited spatial and temporal scope of our sampling highlights the need for further investigation. Future research incorporating broader spatial scales, more detailed habitat characterization, and temporal replication will be essential to disentangle the relative contributions of landscape composition, vegetation structure, and climatic factors shaping parasitoid Hymenoptera diversity in tropical savanna landscapes.

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