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Protocol for collecting Mutillidae (Hymenoptera, Aculeata) in ecological studies: species–area effects on Mutillidae communities

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ABSTRACT

The species–area relationship is one of the most consistent patterns in ecology, and fragmentation is a major cause of habitat loss. Environmental changes in a site can affect the spatial distribution of organisms. Knowledge of Mutillidae ecology is still scarce due to the lack of standardized sampling. Our aim was to: (1) determine the effect of habitat fragmentation on the Mutillidae community and (2) establish a standard method for sampling Mutillidae in ecological studies. Sampling was conducted in four fragments of Brazilian Savanna in an urbanized matrix. We used quadrats with different areas: 25 m^2, 100 m^2 and 400 m^2 to verify sampling effort. Male and female Mutillidae were collected from each of these three treatments. Males were collected using Malaise traps while females were collected through active search. Ecological index, richness, abundance, and percent similarity between fragments were used to analyze the communities. The Kruskal–Wallis test was performed to verify differences between treatments. Non-parametric multivariate analysis of variance was used to determine community composition. Analysis of direct ordination of community with respect to the sample area size was performed. Three hundred individuals were collected; of which 201 were female, 99 male; belonging to 42 species distributed in 13 genera and two subfamilies. The richness, abundance and composition of the community were different between treatments. It was found that a 100 m^2 quadrat was sufficient for comparison and application of ecological concepts and theories for the group.

Introduction

One of the current factors that contribute to the loss of biodiversity is habitat loss and fragmentation (Caughley, 1994; Turner, 1996; Chapin et al., 2000; Debinski and Holt, 2000; Fahrig, 2003). Fragmentation is considered the breaking-up of a continuous habitat and the conversion of it to another type of habitat (Fahrig, 2003). The species–area relationship (Arrhenius, 1921) is one of the best patterns reported among ecological theories (Caley and Schluter, 1997). However, for insects, the species–area relationship is not clear and therefore a no-influence, positive influence, or a no-relation influence may exist (Tscharntke et al., 2002; Ribas et al., 2005; Crist et al., 2006; Brosi, 2009; Gould et al., 2013). There are two main theories that attempt to explain how the species–area relationship is maintained: the theory of island biogeography (MacArthur and Wilson, 1967) and metapopulation (Hanski, 1994, 1998). Habitat fragmentation is one of the forces acting on the mechanisms that control populations and communities (Driscoll, 2008) and fragmentation can be a factor that increases or decreases a population, altering the density of individuals in an area (McKinney, 2006; Su et al., 2015). Environmental variations in a site can also vary the spatial distribution patterns of a species (Aranda and Graciolli, 2015). Due to these environmental variations, sampling methods can further influence the perception of these patterns (Pielou, 1966; Gadagkar et al., 1990; Hernandez et al., 2006; Lavorel et al., 2007; Fraser et al., 2008; Teasdale et al., 2013).

Hymenoptera display a wide range of behaviors and evolutionary strategies, with species that are predators (e.g. Crabronidae, Sphecidae, Vespidae), pollinators (e.g. Apidae *sensu lato*), parasitoids (e.g. approximately 70% of the families of Hymenoptera), and phytophages that cause galls or leaf mining (e.g. Cynipidae, Eulophidae) (Sharkey and Roy, 2002). Sampling methods for collecting individuals depends on the group being collected and sometimes requires more than one method to achieve a satisfactory sample. The efficacy of sampling methods used in ecological

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studies affects data interpretation and conclusions about natural processes (Fraser et al., 2008; Lavorel et al., 2007; Nemésio and Vasconcelos, 2014).

The hymenopteran family Mutillidae is comprised of parasitoid wasps, which generally attack bees and other wasps (e.g. Crabronidae, Sphecidae and Vespidae). Sexual dimorphism is present in Mutillidae evidenced by wingless females and generally winged males (Brothers, 2006). Several techniques are used to collect Mutillidae, such as Malaise and light traps for males; and pitfall and active search for females.

Due to the sexual dimorphism of these insects, few species have both sexes described and females are more commonly studied (Brothers, 2006). The lack of taxonomic association between males and females has become troublesome in ecological studies. There are few studies focused on Neotropical Mutillidae ecology (Vieira et al., 2015); of which, the most commonly used collection method is active search. Therefore, we cannot compare data from other studies with the present one due to previous studies sampling only part of the community (females) and also duo to their lack of sample standardization. Females are most often used in surveys, because they are more easily collected. Active capture of specimens is the most common method of collection, but has been used without standardization. This makes comparison of ecological publications particularly difficult and prevents the advancement of ecological theories for the group.

This study aimed to verify: (1) the effect of fragmentation on the Mutillidae community; testing the hypothesis that larger fragments will have greater species richness, abundance, and diversity; and (2) establish a standard method for collection of Mutillidae to be used in ecological studies.

Material and methods

Study areas

The Brazilian Savanna biome (Cerrado) is a global hotspot with high diversity of flora and fauna (Klink and Machado, 2005) and there are relatively few studies focused on the dynamics of insect communities; especially with regard to the effect of habitat loss and fragmentation. The Cerrado has various vegetation types, ranging from open areas with a predominance of grasses (Cerrado grassland) to forest type formations (Cerradão). Samples were collected in four Cerrado fragments in a urbanized matrix in the municipality of Campo Grande, Mato Grosso do Sul, between January and December 2012, as follows: Matas do Segredo State Park (Segredo) (20°23′ S, 54°35′ O; 175 ha), Prosa State Park (Prosa) (20°27′ S, 54°33′ O; 128 ha), Private Natural Heritage Reserve of Universidade Federal de Mato Grosso do Sul (UFMS) (20°30′ S, 54°36′ O; 32 ha), and Private Natural Heritage Reserve of Universidade Católica Dom Bosco (UCDB) (20°24′ S, 54°36′ O; 25 ha). The region has a subtropical Aw climate (Alvares et al., 2014) with average rainfall of 1125 mm and temperatures with an average range between 17 °C and 29 °C. All fragments have Cerrado *stricto sensu* vegetation and Cerradão; and both Matas do Segredo state park and Prosa state park have gallery forests. The fragments are located within an urban matrix, with varying degrees of isolation and urban occupation. Meteorological data was acquired from the National Institute of Meteorology based on records from the international airport of Campo Grande (OMM:86810).

Samples

In each vegetation fragment we made a grid with a cell size of one hectare. An area of one hectare was used to minimize variation between samples. We randomly chose 25 cells from the grid

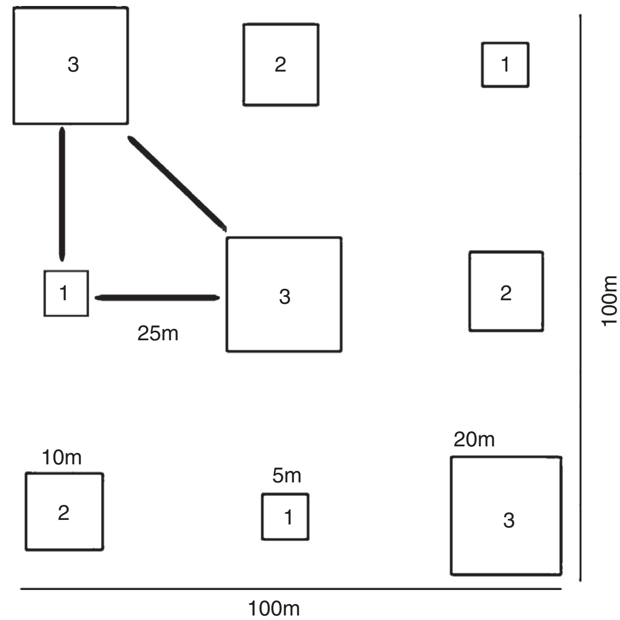

Fig. 1. Diagram of the Latin square model experimental design and arrangement of treatments used for each of the 25 sample points distributed in the four urban fragments.

(Segredo, $n = 8$; Prosa, $n = 6$; UFMS, $n = 6$ e UCDB, $n = 5$) to be sampled sequentially with an average of 14 days between sampling events to accommodate for screening. Mutillidae were collected from quadrats. Three treatments of quadrat were used: 25 m^2, 100 m^2 and 400 m^2. In each sampling cell three quadrats of each treatment were arranged following the experimental design of Latin Squares; this design was used to capture local differences in spatial distribution (Fig. 1) (see Underwood, 1997 for detailed explanation). Each quadrat, independent of their size, was considered a sampling unit totaling 225 sampling units spread over the 25 sampling cells. Sampling was conducted from January to December of 2012.

To collect males, 18 Malaise traps (1.5 m × 1.5 m × 1.0 m) were placed in the sampling cells and distributed amongst the quadrats as follows: one trap placed in each 25 m^2 quadrat, two in each 100 m^2 and three in each 400 m^2. The Malaise traps remained in the quadrats for 72 h (32,400 trap/h). The different numbers of malaise used in each quadrat ware to adjust the sampling effort with respect to the size of the treatment. The short time of trap exposure was compensated by their quantity.

Active search collection was performed from 7:00 to 17:00 to capture females. The amount of time spent collecting females was proportionate to the area of the quadrat used: one hour in 25 m^2, two hours in 100 m^2, and three in 400 m^2. The total effort for active searching of females was 450 man-hours. The specimens collected were labeled and stored in 70% ethyl alcohol. We identified the material using the keys made by Brothers (2006) and confirmed species with specialists. Specimens were deposited in the Zoological Reference Collection of Universidade Federal de Mato Grosso do Sul, Campo Grande (voucher numbers ZUFMSHYM00001 to 000267) and in the entomological collection of the Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil (DZUP) (voucher numbers DZUP 300550-300592).

Statistical analysis

To survey the Mutillidae community in the urban patch's, we use the combined data of each sampled cell, totaling 25 sampled units (Segredo, $n = 8$; Prosa $n = 6$; UFMS, $n = 6$; UCDB, $n = 5$). To test

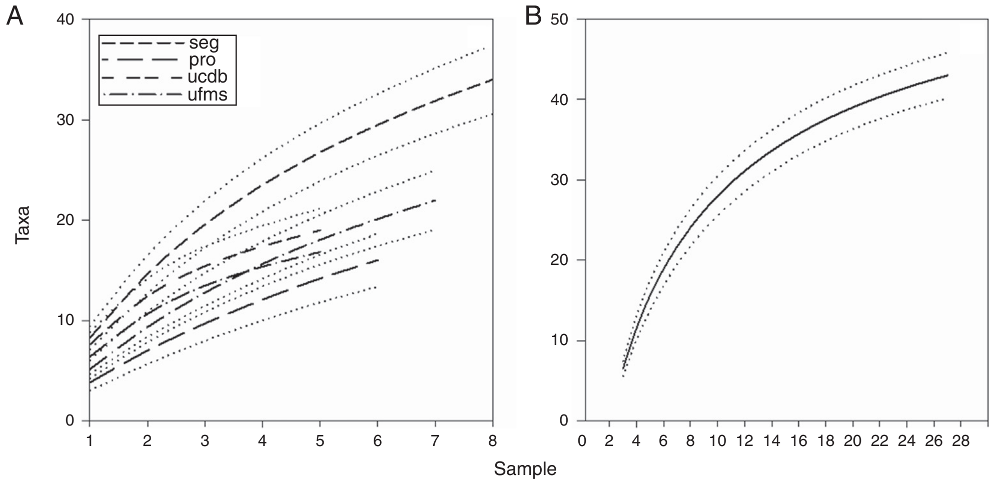

Fig. 2. Rarefaction of Mutillidae species in each fragment (A) and general (B) sampled in Cerrado fragments from January to December 2012.

the standardization of protocol collection, each quadrat was used as sample unit. We used richness estimator JackKnife 1st order, rarefaction, Shannon–Wienner diversity index (H'), evenness Equitability (J'), and Percentage of Similarity (SIMPER, Bray–Curtis) between vegetation fragments to describe communities. For these analyses, all nine quadrats of each sampled cell were used as one sample unit. We used the Rayleigh Test (Z) to verify the differences in seasonality. We tested the normality (Shapiro–Wilk test) of all data collected to determine the use of parametric or non-parametric tests. To verify differences between richness and abundance in treatments we used Kruskal–Wallis test (H) and paired test (post hoc) with Dunn's test. To determine community composition between treatments was used Analyze nonparametric multivariate variance (NPMANOVA) with Bray–Curtis similarity. Among the fragments was performed multivariate analysis of variance (MANOVA) with paired comparison (post hoc) using the Hotelling test. We performed direct ordination analysis of community composition in function with treatment size. All tests used an alpha of 0.05. Statistical analysis was performed in R (vegan packages) (R Core Team, 2013 version 3.2.5) and Past® (Hammer et al., 2001).

Results

We collected 300 specimens, 201 females and 99 males belonging to 42 species, 13 genera and two subfamilies (Table 1). Richness estimate (1st order JackKnife) was 54 species with approximately 78% of fauna being sampled (Fig. 2B). The most common genera were *Ephuta* Say, with 16 morphospecies and 141 specimens; followed by *Traumatomutilla* André, with five species and 28 specimens; and *Darditila* Casal with four species, 28 specimens. We observed a decrease in richness (Female: $Z = 3.91$, $p < 0.05$; Male: $Z = 9.23$, $p < 0.05$) and abundance (Female: $Z = 5.43$, $p < 0.05$; Male: $Z = 46.0$, $p < 0.05$) in the driest and coldest months (June to September) with an increase at the beginning of the rainy season (October) (Fig. 3).

The species richness for each fragment was as follows: Segredo had 33 species ($H' = 3.14$; $J' = 0.89$) followed by UFMS (22 species, $H' = 2.83$; $J' = 0.91$); UCDB (19 species. $H' = 2.64$; $J' = 0.90$); and the Prosa (16 species, $H' = 2.44$; $J' = 0.88$). Overall, there was 85.94% similarity of species among fragments; however the community composition varied (MANOVA: Pillai Trace = 0.7078, $F(1,314) = 51.81$, $p = 0.02$), with the most distinct compositions being Segredo and UFMS (Hotelling test, $p = 0.03$) (Fig. 4).

Table 1
List of Mutillidae species in Cerrado fragments in the municipality of Campo Grande, Mato Grosso do Sul collected between January and December 2012. Locality: 1 – Prosa; 2 – Segredo; 3 – UFMS; 4 – UCDB.

Species	Sex	Locality
Sphaerophtalminae		
Calomutilla aff. *crucigera* (Burmeister, 1854)	♀♂	1, 2, 3, 4
Darditilla cf. *araxa* (Cresson, 1902)	♀	1, 2, 3, 4
Darditilla sp. 1	♂	1, 2, 3, 4
Darditilla sp. 2	♂	2, 4
Darditilla sp. 3	♂	4
Darditilla sp. 4	♀	2
Hoplocrates decumata Mickel, 1941	♂	2
Hoplocrates monacha (Gerstaecker, 1874)	♀	2, 3
Hoplomutilla alagoa (Cresson, 1902)	♂	3
Hoplomutilla anthracina (Gerstaecker, 1874)	♂	2, 3, 4
Hoplomutilla goyazana (André, 1898)	♀	1
Hoplomutilla myops flavimyops Mickel, 1939	♀	3, 4
Hoplomutilla triumphans Mickel, 1939	♀	4
Lophomutilla obscura Fritz and Pagliano, 1993	♀	2, 3, 4
Lophostigma sp.	♀	2, 3
Lynchiatilla silvai Casal, 1963	♀	2
Pseudomethoca sp. 1	♀	1, 2, 3, 4
Pseudomethoca sp. 2	♂	1, 2, 3
Ptilomutilla pennata André, 1905	♀	1, 2
Sphaeropthalma sp. 1	♂	2
Traumatomutilla geographica (Gerstaecker, 1874)	♀	2
Traumatomutilla spectabilis (Gerstaecker, 1874)	♀	1, 2, 3, 4
Traumatomutilla sp. 1	♀	2
Traumatomutilla sp. 2	♀	2
Traumatomutilla sp. 3	♀	3, 4
Mutillinae		
Ephuta sp. 1	♀	1, 2, 3, 4
Ephuta sp. 2	♀	1, 2, 3, 4
Ephuta sp. 3	♀	1, 2
Ephuta sp. 4	♀	1, 4
Ephuta sp. 5	♀	2
Ephuta sp. 6	♀	2
Ephuta sp. 7	♀	2, 4
Ephuta sp. 8	♀	1, 2
Ephuta sp. 9	♀	3
Ephuta sp. 10	♂	2, 4
Ephuta sp. 11	♂	2, 3
Ephuta sp. 12	♂	2, 3
Ephuta sp. 13	♂	2
Ephuta sp. 14	♂	2, 3, 4
Ephuta sp. 15	♂	1, 2
Ephuta sp. 16	♂	1, 2, 3, 4
Timulla terminalis (Gerstaecker, 1874)	♀	1, 2, 4

Table 2
Kruskal–Wallis test for species richness and abundance of individual Mutillidae and result of the similarity of NPMANOVA test.

Kruskal–Wallis test (*H*)	Species richness			Individual abundance		
	H	*Q*	*p*	*H*	*Q*	*p*
$25\,m^2$ vs. $100\,m^2$	4.616	2.522	0.03*	6.206	4.612	0.01*
$25\,m^2$ vs. $400\,m^2$	5.368	4.782	0.02*	8.885	4.612	0.01*
$100\,m^2$ vs. $400\,m^2$	0.274	2.261	0.60	0.611	1.737	0.43

Non-parametric MANOVA Bray–Curtis SIMPER	*F*	*p*	Similarity (%)
Segredo vs. Prosa	2.578	0.02*	84.50
Segredo vs. Ucdb	1.639	0.10	84.46
Segredo vs. Ufms	2.905	0.02*	88.42
Prosa vs. Ucdb	1.663	0.12	85.59
Prosa vs. Ufms	0.688	0.61	86.77
Ucdb vs. Ufms	1.475	0.16	85.58
General			85.94

H = Kruskal–Wallis test value; *Q*: Dunn's method value; *F*: Fisher test value.
* Statistical difference, $p \leq 0.05$.

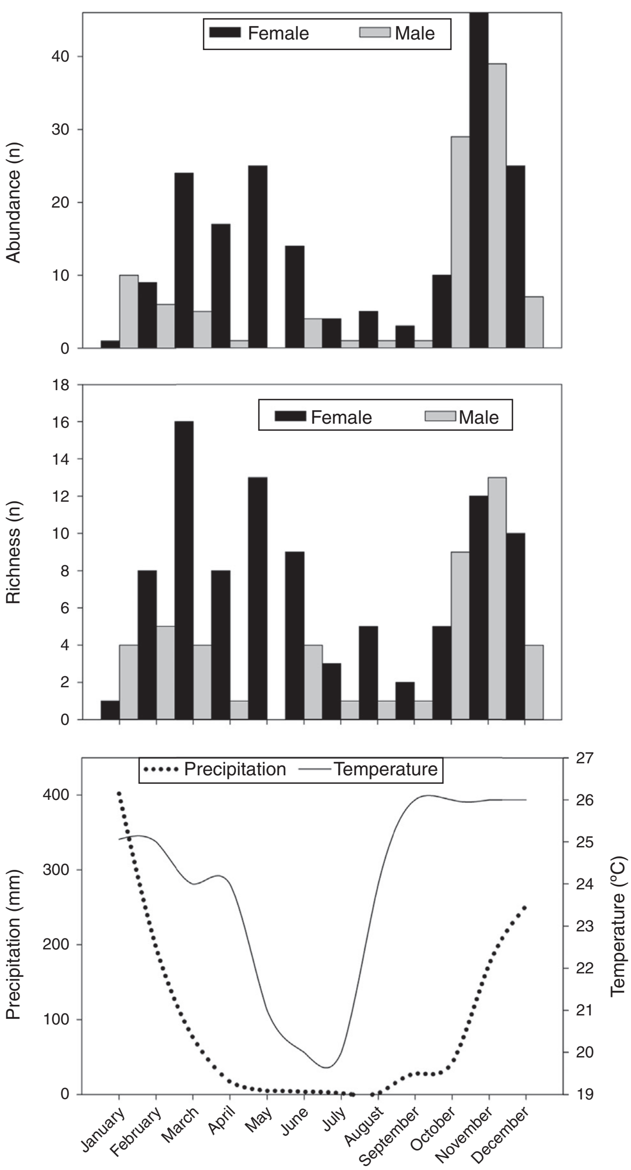

Fig. 3. Monthly distribution of the richness and abundance of male and female Mutillidae and temperature and precipitation variations in Cerrado fragments from January to December 2012.

Between the treatments, there was a significant difference in species richness (treatment: mean ± sd. $25\,m^2$: 1.54 ± 1.03; $100\,m^2$: 2.08 ± 1.2; $400\,m^2$: 2.63 ± 2.15) ($H = 13.68$, d.f. = 2, $p = 0.001$). The abundance of individuals between $100\,m^2$ and $400\,m^2$ was not different (treatment: mean ± sd. $25\,m^2$: 1.73 ± 1.66; $100\,m^2$: 2.5 ± 1.63; $400\,m^2$: 3.53 ± 3.63) ($H = 12.93$ d.f. = 2, $p = 0.002$). For community composition (NPMANOVA, $F = 1.898$, $p < 0.01$) (Table 2) differences in treatments using $25\,m^2$ and $400\,m^2$ quadrats were observed. We could see the structure of the community in terms of the size of the sample area through direct ordering with a $100\,m^2$ quadrat containing enough species to represent the community (Fig. 5).

Discussion

Fragmentation in community composition

The last survey of Mutillidae from the state of Mato Grosso do Sul recorded 83 species (Luz et al., in press). In this study we observed 50.6% of the entire state's species richness. Of the 42 species recorded in this work, eight have never been recorded in Mato Grosso do Sul and are: *Calomutilla aff. crucigera* (♀♂) (Burmeister, 1854), an undescribed species of *Darditilla* (♀), *Hoplocrates decumata* (♂) Mickel, 1941, *Hoplomutilla alagoa* (♂) (Cresson, 1902), *H. anthracina* (♂) (Gerstaecker, 1874), *Lophomutilla obscura* (♀) Fritz and Pagliano, 1993, *Lynchiatilla silvai* (♀) Casal, 1963, an undescribed species of *Pseudomethoca* (♂), and *Lophostigma* (♀). The fact that new species are being recorded from urban vegetation fragments shows how scarce knowledge about this family is, this is typically the case of Neotropical and Brazilian Savanna fauna. *Traumatomutilla*, *Darditilla*, and *Hoplomutilla* Ashmead are highly diverse genera in the Neotropical region (Nonveiller, 1990) and were most abundant in our study. *Ephuta*, a diverse and poorly known genus, presented many undefined species in this work. With a lack of taxonomic description and sexual association for *Ephuta*, we may have overestimated the species number for this genus. Moreover, *Timulla* Ashmead which is widely distributed and has high species diversity, only presented a single species in this study.

We observed a temporal variation in richness and abundance; both increased in the beginning of the rainy season (October to December) for males and females. At the end of the rainy season (March to May), a second increase was observed only for females (Fig. 2). The temporal variation in richness and abundance does not affect the comparison between treatments, since the sampling is done simultaneously, the variation of ecological metrics occurred similarly in treatments. This pattern is likely a response to the availability of hosts (resources), which also are in great abundance

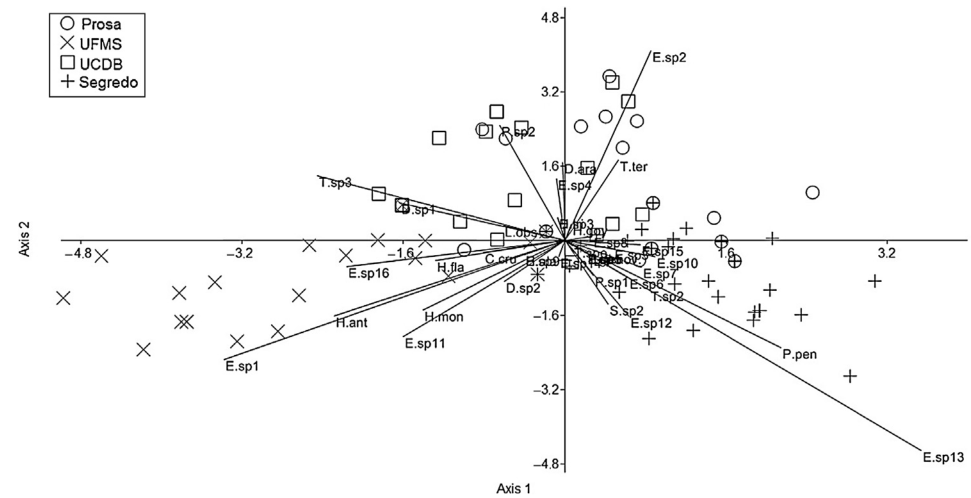

Fig. 4. Canonical Variable Analysis (CVA) from the multivariate analysis of variance (MANOVA) of the species composition of Mutillidae between the Cerrado fragments (Pillai Trace = 0.7078, $F(1.314) = 51.81$, $p = 0.02$). Matas do Segredo State Park (Segredo), Prosa State Park (Prosa), Private Natural Heritage Reserve of Universidade Federal de Mato Grosso do Sul (UFMS) and Private Natural Heritage Reserve of Universidade Católica Dom Bosco (UCDB).

during these periods (Hawkins, 1994; Lewis et al., 2002; Tylianakis et al., 2005; Shaw, 2006; Christie et al., 2009; Harvey et al., 2009; Norden et al., 2009; Polidori et al., 2009; Kidd and Amarasekare, 2012).

Males were more abundant at the beginning of the rainy season, which is the putative breeding season. After this period, there is a gradual decrease in male abundance due to death after copulation (Tormos et al., 2009). Females exhibit a decrease in abundance after

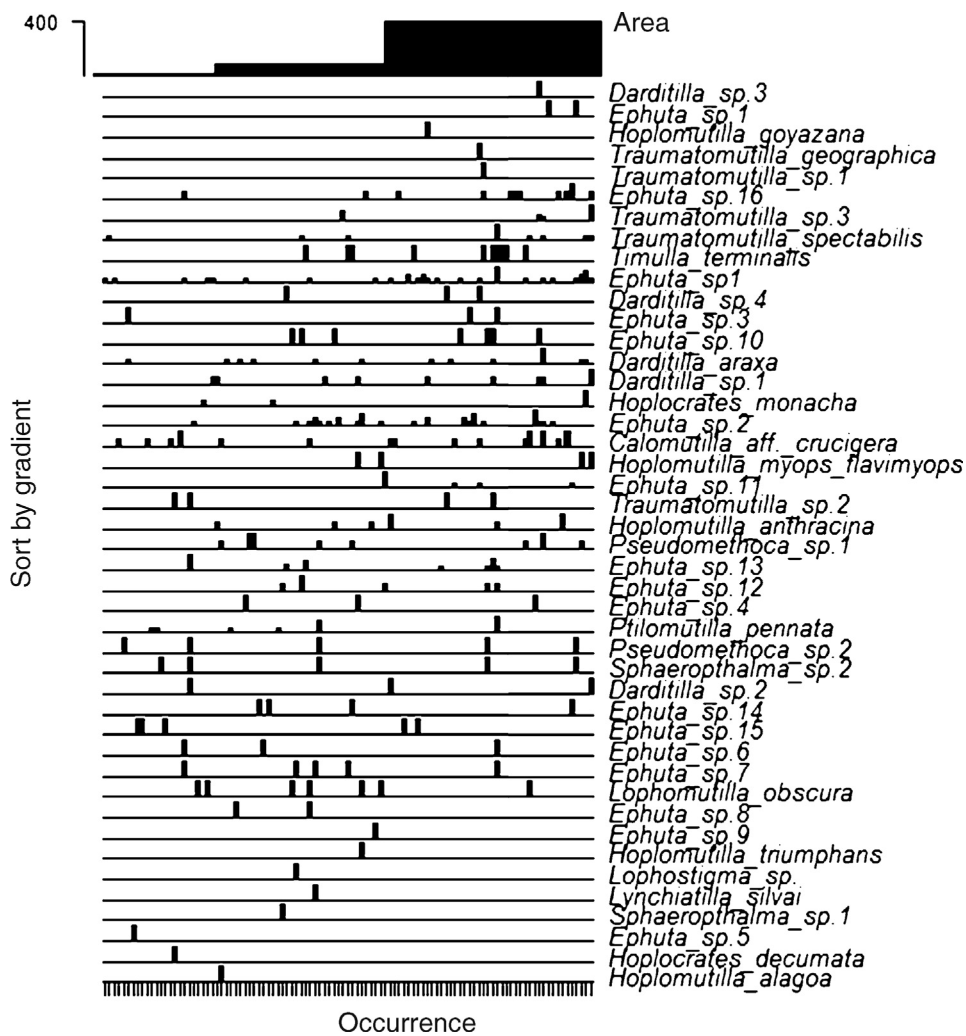

Fig. 5. Direct ordering analysis of Mutillidae community in relation to the sample area gradient.

the second peak, before the dry season. The community reestablishes itself due to the emergence of individuals that were pupae during the dry season.

With respect to fragment size, we observed a pattern of higher diversity in larger fragments, except for Prosa State Park, the second largest in area, which had the lowest values of diversity and evenness. The vegetation type of Prosa State Park is dominated by seasonal and gallery forests, which differs from the other vegetation fragments; which are mostly Cerrado. Mutillidae is known to be more abundant and diverse in drier areas (Brothers et al., 2000; Brothers, 2006) being strongly influenced by host availability (Aranda and Graciolli, unpubl. data) and this is significantly reflected in community composition.

The variation in community composition differed between the fragments. Most species were similar among the fragments; however, the fragment from UFMS was the most different from the others mainly by the occurrence of *Hoplocrates monacha* (Gerstaecker, 1874), *H. anthracina*, *H. myops flavimyops* Mickel, 1939, and many *Ephuta* spp. The edge effect is an important factor influencing the Mutillidae community (Vieira et al., 2015), with fragment size, degree of insulation, and surrounding characteristics modulating the community structure. For the fragments in this study, the spatial distribution of Hymenoptera fauna varied only with respect to vegetative structure (Aranda and Graciolli, 2015).

Defining collection protocol

In Mutillidae, we observed an effect of fragmentation on the community structure and we highlight the influence of the size of sampling area, when sampling the group. Mutillidae females can roam large areas in search of hosts. Diurnal species, which comprises the majority of Mutillidae species, stop overnight in temporary locations (Bergamaschi et al., 2011, 2012). Thus, because of their solitary and host searching behavior, the collection methods for Mutillidae are mostly based on active search; however, for collections applied to ecological studies there are still no defined protocols.

Malaise traps and pan traps trays are efficient for collecting males, but they are unrepresentative of the Mutillidae community. Short collection periods show low amounts of Mutillidae and cannot be used to represent the fauna of this family with certainty; as can be seen in studies that use these techniques and always report low mutillid frequencies (Azevedo and Santos, 2000; Azevedo et al., 2002, 2003; Souza et al., 2006; Alencar et al., 2007; Feitosa et al., 2007), which are not necessarily the norm for this family (Schmidt and Buchmann, 1986).

Standardizing the sampling effort for space and time, we see that the differences in community structures are caused by the size of the sampled area. The treatment of 25 m^2 showed significant differences in richness, abundance and composition of the community when compared to the other treatments. We observed that collection data has a diminishing return based on quadrat size; this is justified by the observation that the 25 m^2 quadrat had only two unique species when compared to the 100 m^2 size, and the 400 m^2 had only five (Fig. 5). The 100 m^2 and 400 m^2 quadrats showed similar richness, abundance, and species composition, both of which significantly greater than the 25 m^2 quadrats. Thus, the community representation is not impaired by sampling units of 100 m^2. When defining sampling effort as area per time we have a valid sampling unit for comparison and application of ecological concepts and theories for the group, with 100 m^2 being a valid unit. Considering the temporal variation in abundance of Mutillidae, the best time for sampling individuals of this family in the Cerrado region is during the rainy season, due to its marked seasonality, with a rainy season in summer and a dry season in winter.

Since there are few ecological studies focused on this family, it is important to define and establish sampling methods for collecting specimens. The methods proposed herein are shown to be valid for comparisons and applications of ecological concepts and theories for this group. We advise the use of 100 m^2 quadrats to maximize sampling efforts. We expect that the standardization of field collections can improve future research toward better understand aspects of the ecology of this group.

Conflicts of interest

The authors declare no conflicts of interest.

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