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Population survey of dung beetles (Coleoptera: Scarabaeidae) in a conservation unit in Eastern Maranhão

Levantamento populacional de besouros coprófagos (Coleoptera: Scarabaeidae) em uma unidade de conservação do Leste do Maranhão

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Abstract: The order Coleoptera varies greatly in shape, size, ecological strategy, and habitat. Coprophagous beetles belong to the superfamily Scarabaeoidea, family Scarabaeidae, and subfamily Scarabaeinae and are commonly known as dung beetles. The main objective of this study was to carry out a population survey of dung beetles (Coleoptera: Scarabaeidae) in a conservation unit in eastern Maranhão State, Brazil. The experimental area was divided into three sections: road verge (Area 1), Cerrado *sensu stricto* (Area 2), and riparian forest (Area 3). Two collections were carried out, one in October 2020 and one in May 2021. Sampling was performed using pitfall traps, which were kept in the field for 4 days (96 h). Statistical analyses were performed using ANAFAU software. Analyses of species richness and accumulation curves were performed using EstimateS software. A total of 393 Scarabaeidae specimens were collected, of which 145 individuals, distributed into 8 species, were from Area 1, 149 individuals, distributed into 8 species, were from Area 2, and 99 individuals, distributed into 8 species, were from Area 3. All areas had the same observed species richness, with 8 species each. The species diversity of the three studied phytophysiognomies was analyzed using the Shannon–Wiener index. Area 3 had the highest diversity index, which was confirmed by Pielou's evenness index (*J'*). Richness estimators and accumulation curves revealed that the sampling effort was not sufficient to quantify dung beetle species in any of the three areas. Therefore, the richness of Scarabaeidae is undersampled in the Inhamum Environmental Protection Area.

Keywords: Inhamum environmental; Protection area; Abundance; Cerrado; Dung beetles.

Resumo: A ordem Coleóptera possui uma grande variedade de formas, tamanhos, estratégias ecológicas e habitats. Os coleópteros coprófagos pertencem a superfamília Scarabaeoidea, família Scarabaeidae, subfamília Scarabaeinae e são conhecidos popularmente como rola-bosta. O objetivo principal do presente trabalho foi realizar o levantamento populacional dos besouros coprófagos (Coleoptera: Scarabaeide) em uma Unidade de Conservação do Leste do Maranhão. A área experimental em estudo foi dividida de três formas: Margem da estrada (borda); Cerrado senso stricto e Mata ciliar. Para realização das coletas dos besouros coprófagos, foram realizadas duas coletas, uma no mês de outubro (2020) e outra no mês de maio de (2021), utilizando armadilhas do tipo Pitifull. Para as análises estatísticas utilizou-se o programa ANAFAU. Para a análise da riqueza e curva de acumulação utilizou o programa EstimateS. Foram contabilizados 393 espécimes de Scarabaeidae, sendo 145 indivíduos para a Área 1 distribuídos em oito espécies, 149 indivíduos para a Área 2 distribuídos em oito espécies e 99 indivíduos para a Área 3 distribuídos em oito espécies, sendo obtida igualdade de riqueza observada de espécies para todas as áreas com oito espécies respectivamente. Foi analisada a diversidade das espécies entre as três fitofisionomias estudadas (Shannon-Wiener), a Área 3 teve maior índice de diversidade, sendo confirmado pelo Índice de Pielou (J). O estimador de riqueza e as curvas de acumulação obtidas para ambas as áreas revelaram que o esforço amostral não foi suficiente para quantificar totalmente as espécies e que, portanto, a riqueza de Scarabaeidae da APA do Inhamum encontra-se subamostrada.

Palavras-chave: APA do Inhamum; Proteção da área; Abundância; Cerrado; Rola-Bosta.

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1. Introduction

The Cerrado biome has experienced constant degradation of its natural ecosystems, owing to the establishment of agricultural fields, creation of transportation routes, forest fires, and lumber extraction, among other factors. Such ecosystem changes have direct impacts on biological communities, as evidenced by the reported alterations in floristic and faunal structures (PRIMACK et al., 2001). Studies predict that, if deforestation continues at the current pace, by 2050, the Cerrado biome will have suffered the largest species extinction in world history. One of the main causes of these disturbances is the fragmentation of natural ecosystems, with a subsequent loss of species diversity (PRIMACK et al., 2001). In addition to having biological implications for agricultural areas, ecosystem fragmentation increases the rate of erosive processes that transport sediments to water bodies, affecting water quality and promoting the siltation of rivers and reservoirs.

Forest fragmentation not only influences the richness and composition of invertebrates but also modifies higher-order interactions between arthropods and other organisms, given that these animals are sensitive to subtle variations in their habitats (DIDHAM et al., 1996). Among representatives of the phylum Arthropoda, insects are considered good indicators of environmental impact, as they have a great diversity of taxa and habitats and exert a strong influence on biological processes in natural ecosystems (WINK et al., 2005).

The order Coleoptera includes organisms with a wide variety of shapes, sizes, ecological strategies, and habitats. Its representatives are found on all continents, except Antarctica. It is the most diverse order of the class Insecta, with about 360,000 species distributed in approximately 180 families (BOUCHARD et al., 2009; BOUCHARD et al., 2011). With rapid responses to anthropogenic pressures on natural environments (TEIXEIRA et al., 2009), coleopterans serve as bioindicators of environmental quality and ecosystem conservation. Their value as bioindicators stems from their high degree of ecological niche specialization, high taxonomical and ecological diversity, easily collection in large quantities, and functional importance to ecosystems (CARVALHO, 2011; CASARI, 2012; WINK et al., 2005). Furthermore, coleopterans are well-known systematically and biologically and have a wide geographical distribution (DAJOZ, 2005).

Coprophagous beetles belong to the superfamily Scarabaeoidea, family Scarabaeidae, and subfamily Scarabaeinae and are commonly known as dung beetles. The Scarabaeoidea are found in almost all biomes worldwide, being represented by about 20,000 species (RONQUI; LOPES, 2006). Neotropical scarabaeoid communities have been increasingly affected by anthropogenic impacts, habitat changes, and ecosystem fragmentation (NICHOLS et al., 2007). It is important to perform comparative studies of local fauna in order to understand anthropogenic impacts on biological diversity (HUMPHREY et al., 1999; HUTCHESON; JONES, 1999). Given the above, this study aimed to conduct a population survey of dung beetles (Coleoptera: Scarabaeidae) in a conservation unit in eastern Maranhão State, Brazil.

2. Methods

2.1 Location and characterization of the study area

The study was conducted in the Inhamum Municipal Environmental Protection Area (EPA), located on the left bank of BR-316, near the urban perimeter of Caxias (4°53′30″S 43°24′53″W) (ALBUQUERQUE, 2012). The region has dry subhumid climate with two well-defined seasons, a rainy season from December to June and a dry season from July to November (ALBUQUERQUE, 2012). Rainfall ranges from 1600 to 1800 mm annually, and the minimum, average, and maximum temperatures are commonly high, with the annual average above 24 °C (ARAÚJO, 2012).

2.2 Experimental areas and phytophysiognomies

Collections were performed in three areas with distinct phytophysiognomies in the Inhamum EPA, as follows: road verge (Area 1), Cerrado *sensu stricto* (Area 2), and riparian forest (Area 3). The road verge area was sampled at a distance of 10 m from the edge of the road. This open area is characterized by the presence of shrubs, grasses, and scattered trees. The Cerrado *sensu stricto* area (250 m from the first transect of the road edge) has a typical cerrado vegetation with small- and medium-sized trees and large quantities of grasses and shrubs. The riparian forest area (500 meters from the first transect of Cerrado *sensu stricto*) has denser vegetation characterized by small, medium, and large trees with open and semi-open canopies. The area is well shaded and contains large amounts of leaf litter.

2.3 Specimen collection and identification

In each experimental area, three parallel transects were set up approximately 20 m apart. Then, five equidistant sampling units (20 × 20 m) were marked along each transect, totaling 15 points per area and 45 points overall (Figure 1).

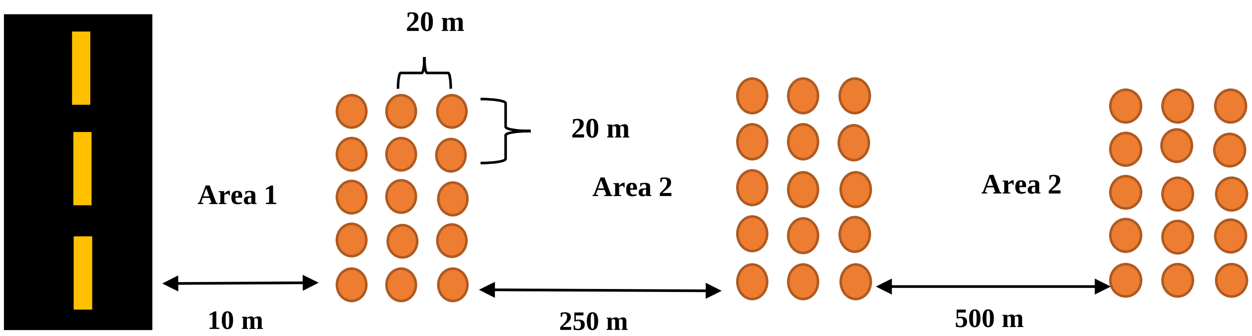

Figure 1 – Schematic of the distribution and spacing of pitfall traps in each study area.
Source: Authors (2021).

Pitfall traps were used for the sampling of dung beetle individuals. Each trap consisted of a 500 mL plastic cup containing 5% formalin and 20 mL of detergent to reduce surface tension. Traps were buried at ground level. A bait was placed inside a 50 mL plastic pot, which was hung above the buried cup. A plastic dish was used to protect traps from environmental conditions. Each trap was kept in the field for 96 h (4 days).

After 96 h, traps were removed from the field and identified with the date and place of collection. Then, they were transported to the Soil Fauna Laboratory (LAFS), CESC, Maranhão State University, where the contents were washed over a 0.25 mm sieve and transferred to plastic flasks containing 70% ethanol. Individuals were separated, identified, and counted (BORROR; DELONG, 1969; COSTA et al., 2006) by using entomological forceps and a Zeiss Stemi DV4 stereomicroscope. Collected beetles were identified at the species level using the dichotomous key of Silva, Vaz-de-Mello, and Di Mare (2011).

2.4 Data analysis

For statistical analysis, a database was initially created using a Microsoft Excel spreadsheet, from which a table was constructed to depict the species composition of dung beetle populations of the three areas. Statistical analyses were performed using two software. Faunal analyses based on dominance, abundance, frequency, Shannon–Weaner diversity index (*H′*), and Pielou's evenness (*J′*) indices were performed using ANAFAU software (MORAES et al., 2003). Richness and accumulation curve analyses were performed using EstimateS software (COLWELL, 2013).

3. Results and discussion

3.1 Abundance, frequency, constancy, and dominance of Scarabaeidae species

A total of 393 specimens of Scarabaeidae were sampled, with 145 individuals in Area 1, 149 individuals in Area 2, and 99 individuals in Area 3. Specimens were classified into 9 genera and 12 species. Each area was found to have an observed richness of eight species (Table 1). The greater abundance of individuals in Areas 1 (road verge) and 2 (Cerrado *sensu stricto*) compared with Area 3 (riparian forest) might be related to phytophysiognomy. The first two areas are characterized by open vegetation, which facilitates the entry of mammalians, leading to greater availability of food resources (dung and carrion) for dung beetles. Thus, environments with greater diversity of mammals and other small and large vertebrates provide more food resources for scavenger dung beetles. Changes in the mammalian community lead to changes in the richness and abundance of Scarabaeidae (ESTRADA et al., 1998; ANDERSON; LAURANCE, 2007).

In Area 1, *Dichotomius nisus* was superabundant, with 91 individuals (62.76%), and *Canthon chalybaeus* was very abundant (14.50%) (Table 1). *Dichotomius nisus* was superdominant, *Ateuchus* sp., *Canthon chalybaeus*, and

Pseudocanthon xanthurus were very dominant, *Dichotomius nisus* was super frequent, and *Canthon chalybaeus* was very frequent. Constancy analysis showed that *Ateuchus* sp., *Canthon chalybaeus*, *Deltochilum granulosum*, *Dichotomius nisus*, *Dichotomius sericeus*, *Ontherus sulcator, Pseudocanthon xanthurus*, and *Uroxys* sp. were constant. (Table 1). *Dichotomius* sp. and *Uroxys* sp. were exclusive to Area 1 (Table 1).

Table 1 – List of taxonomic species collected from Areas 1 (road verge), 2 (Cerrado sensu stricto*, 250 m from the road verge), and 3 (riparian forest, 500 m from the first Cerrado transect), number of individuals (NI), number of collections (NC), relative percentage, abundance (A), dominance (D), frequency (F), and constancy (C) in the Inhamum Environmental Protection Area, Caxias, Maranhão, Brazil.*

	AREA 1							AREA 2							AREA 3						
SPECIES	**NI**	**%**	**NC**	**A**	**D**	**F**	**C**	**NI**	**%**	**NC**	**A**	**D**	**F**	**C**	**NI**	**%**	**NC**	**A**	**D**	**F**	**C**
Ateuchus sp	10	6,70	1	c	D	F	W	17	11,40	2	va	D	VF	W	28	28,28	2	va	D	VF	W
Canthidium sp	-	0,00	-	-	-	-	-	17	11,40	1	va	D	VF	W	8	8,08	1	c	D	F	W
Canthon chalybaeus	21	14,50	1	va	D	VF	W	11	7,38	2	c	D	F	W	2	2,02	1	r	ND	IF	W
Canthon sp	-	0,00	-	-	-	-	-	1	0,70	1	r	ND	IF	W	4	4,04	1	d	ND	IF	W
Deltochilum granulosum	2	1,37	1	d	ND	IF	W	3	2,02	1	r	ND	IF	W	-	0,00	-	-	-	-	-
Dichotomius nisus	91	62,76	2	va	SD	SF	W	81	54,36	2	sa	SD	VF	W	17	17,17	1	c	D	F	W
Dichotomius sp	4	2,75	1	c	ND	F	W	-	0,00	-	-	-	-	-	-	0,00	-	-	-	-	-
Eurysternus caribaeus	-	0,00	-	-	-	-	-	-	0,00	-	-	-	-	-	17	17,17	2	c	D	F	W
Ontherus sulcator	2	1,37	2	d	ND	IF	W	6	4,02	1	c	D	F	W	-	0,00	-	-	-	-	-
Ontherus sp	-	0,00	-	-	-	-	-	-	0,00	-	-	-	-	-	1	1,02	1	r	ND	IF	W
Pseudocanthon xanthurus	11	7,60	1	c	D	F	W	13	8,72	2	c	D	F	W	22	22,22	1	va	D	VF	W
Uroxy sp	4	2,75	1	c	ND	F	W	-	0,00	-	-	-	-	-	-	0,00	-	-	-	-	-
TOTAL	**145**	**100**						**149**	**100**						**99**	**100**					

Analyses were performed using ANAFAU software. Abundance classes: (sa) superabundant; (va) very abundant; (a) abundant; (c) common; (d) disperse; (r) rare. Dominance classes: (sd) superdominant; (d) dominant; (nd) non-dominant. Frequency classes: (sf) super frequent; (vf) very frequent; (f) frequent; (if) infrequent. Constancy classes: (w) constant.

Source: Authors (2021).

In Area 2, *Dichotomius nisus* was superabundant, with 81 individuals (54.36%), and *Ateuchus* sp. (11.40%) and *Canthidium* sp. (11.40%) were very abundant. *Dichotomius nisus* was superdominant, *Ateuchus* sp., *Canthidium* sp., *Canthon chalybaeus*, *Ontherus sulcator*, and *Pseudocanthon xanthurus* were dominant, *Dichotomius nisus* was super frequent, and *Ateuchus* sp. and *Canthidium* sp. were very frequent. Constancy analysis showed that *Ateuchus* sp., *Canthidium* sp., *Canthon chalybaeus*, *Canthon* sp., *Deltochilum granulosum*, *Dichotomius nisus*, *Ontherus sulcator*, and *Pseudocanthon xanthurus* were constant. Area 2 did not have exclusive species (Table 1).

In Area 3, the very abundant species were *Ateuchus* sp., with 28 individuals (28.28%), and *Pseudocanthon xanthurus*, with 22 individuals (22.22%). The common species were *Canthidium* sp. (8.8%), *Dichotomius nisus* (17.17%), and *Eurysternus caribaeus* (17.17%) (Table 1). The following species were identified as dominant: *Ateuchus* sp., *Canthidium* sp., *Dichotomius nisus*, *Eurysternus caribaeus*, and *Pseudocanthon xanthurus*. The non-dominant species were *Canthon chalybaeus*, *Canthon* sp., and *Ontherus* sp. The most common species was *Ateuchus* sp., and the frequent species were *Canthidium* sp., *Dichotomius nisus*, and *Eurysternus caribaeus*. *Ateuchus* sp., *Canthidium* sp., *Canthon chalybaeus*, *Canthon* sp., *Dichotomius nisus*, *Eurysternus caribaeus*, *Ontherus* sp., and *Pseudocanthon xanthurus* were classified as constant (Table 1). *Eurysternus caribaeus* and *Ontherus* sp. were exclusive to Area 3 (Table 1).

The high abundance of *Dichotomius nisus* in Areas 1 and 2 might be related to the greater amount of food resources, such as mammalian excrement, found more frequently in these two phytophysiognomies. Another factor that might have contributed to the greater abundance of *Dichotomius nisus* in Areas 1 and 2 is the type of vegetation, given that *Dichotomius nisus* is a nocturnal tunneler, occurring in the Cerrado, Caatinga, and Pantanal regions of Brazil. The species may be found on river banks, forest edges, areas with a high degree of disturbance, and pastures, where it is dominant (FARIAS; HERNANDES, 2017; VAZ-DEMELLO et al., 2017).

Dichotomius sp. was exclusive to Area 1, probably because the region is degraded compared with the other areas. Ramos et al. (2010) reported that the presence of *Dichotomius sp*. indicates degraded or open areas. Thus, the exclusivity of these individuals might be related to the open environment and high degradation of Area 1. Another species that was exclusive to Area 1 was *Uroxys*. According to EMBRAPA (2019), *Uroxys*, a dark-brown beetle, is found in several Brazilian states and has endocoprid behavior, generalist feeding habit, and preference for arboreal forests. Koller et al. (2007) found that *Uroxys* is likely resistant to different environments. Thus, the exclusivity of this species in Area 1 (road verge) might be related to its likely resistance to a variety of environments.

Ateuchus sp. was the most abundant in Area 3 (Table 1), which might be related to the high availability of food resources in the area. Area 3 has a more conserved forest environment than Areas 1 and 2. According to Vaz-de-Mello (1999), the genus *Ateuchus* comprises about 100 species. Most species are copronecrophagous, occurring in forested areas of the Neotropical region. Another factor that might have contributed to the species being the most abundant in Area 3 was the type of material used in traps (cattle manure). The preference of *Ateuchus sp*. for cattle manure was also reported by Audino (2007), who found that these individuals were caught in greater numbers in traps baited with bovine manure.

Eurysternus caribaeus and *Ontherus sp.* were exclusive to Area 3. The tribe Eurysternini was created by Vulcano et al. (1960) solely to house the genus *Eurysternus* (MARTINEZ, 1987), given the differences in morphology and feeding and nesting behavior. The genus comprises species with diurnal and nocturnal habits, occurring in forests or woods with low luminosity and high humidity. Thus, the exclusivity of this species in Area 3 is related to environmental conditions. The area is characterized by a more humid climate, milder soil temperatures, and a more forested environment in comparison with the other phytophysiognomies. Furthermore, the area contains large trees that provided constant shade to the collection points, favoring the sampling of these individuals.

3.2 Shannon diversity index (*H′*) and Pielou evenness (*J′*)

Diversity is a fundamental concept in the study of communities, and there are several methods available for its measurement, including the use of diversity indices that combine two attributes of a biological community, namely number of species and their evenness (MELO et al., 2008). We determined the diversity and evenness indices for Areas 1, 2, and 3 (Table 2).

Table 2 – Shannon–Wiener diversity (H') and Pielou evenness (J') indices of Scarabaeidae collected from Areas 1 (road verge), 2 (Cerrado sensu stricto, 250 m from the road verge), and 3 (riparian forest, 500 m from the first Cerrado transect).

Index	Area 1	Area 2	rea 3
Shannon-Wiener (H’)	1.6334	1.7181	1.7547
Uniformity or Equability (J)	0.8394	0.8829	0.8438

Source: Authors (2021).

Area 3 had the highest diversity index (H' = 1.7547), followed by Area 2 (H' = 1.7181) and Area 1 (H' = 1.6334). The diversity indices of Areas 2 and 3 were similar, probably associated with the phytophysiognomy of the areas, characterized by high amounts of trees and shrubs compared with Area 1. The openness and lack of large and medium-sized trees likely influenced the low diversity of Area 1. Similar results were observed by Marcon (2011) and Silva (2011), who analyzed Scarabaeinae diversity in three distinct areas with good, intermediate, and poor states of conservation. It was observed that Shannon diversity was higher in the most conserved areas. Thus, the higher diversity indices for Areas 2 and 3 might be related to their forested environments.

A high Pielou evenness index indicates high diversity, that is, that all species are equally abundant. Area 2 (J' = 0.8829) had the highest evenness, followed by Area 3 (J' = 0.8438) and Area 1 (J' = 0.8394).

3.3 Estimated richness (*S*) and species accumulation curve

In all areas, the observed richness was 8. The jackknife 1 estimator revealed an estimated richness of 14 species in Area 1, 9 species in Area 2, and 10 species in Area 3 (Table 3). These findings indicate that the sampling effort was not sufficient to fully quantify the species; therefore, the Scarabaeidae community was undersampled, and it is possible to find even greater richness in the three phytophysiognomies (Table 3).

Area 3 had the highest diversity index (H' = 1.7547), followed by Area 2 (H' = 1.7181) and Area 1 (H' = 1.6334). The diversity indices of Areas 2 and 3 were similar, probably associated with the phytophysiognomy of the areas, characterized by high amounts of trees and shrubs compared with Area 1. The openness and lack of large and medium-sized trees likely influenced the low diversity of Area 1. Similar results were observed by Marcon (2011) and Silva (2011), who analyzed Scarabaeinae diversity in three distinct areas with good, intermediate, and poor states of conservation. It was observed that Shannon diversity was higher in the most conserved areas. Thus, the higher diversity indices for Areas 2 and 3 might be related to their forested environments.

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Table 3 – Richness estimates for Scarabaeidae species collected in the Inhamum Environmental Protection Area, Caxias, Maranhão, Brazil, in October 2020 and May 2021.

Estimator	Area 1	Area 2	Area 3
Observed richness	8	8	8
Jackknife 1	14	9	10

Source: Authors (2021).

Figure 2 shows that, for all areas, the species curves did not reach an asymptote, although the richness values were close to those provided by Jackknife 1. A greater sampling effort is necessary to reach the asymptote. According to Colwell (2004), the accuracy of richness estimators increases with increasing sample size. In studies on animals with high numbers of individuals per sampling, such as arthropods, diversity is best evaluated using richness estimators (DIAS, 2004). Species richness of discovered taxa is as important as the number of species to be discovered in ecological conservation programs (SANTOS, 2003), and richness and diversity data are essential to subsidize conservation policies (CODDINGTON et al., 1991).

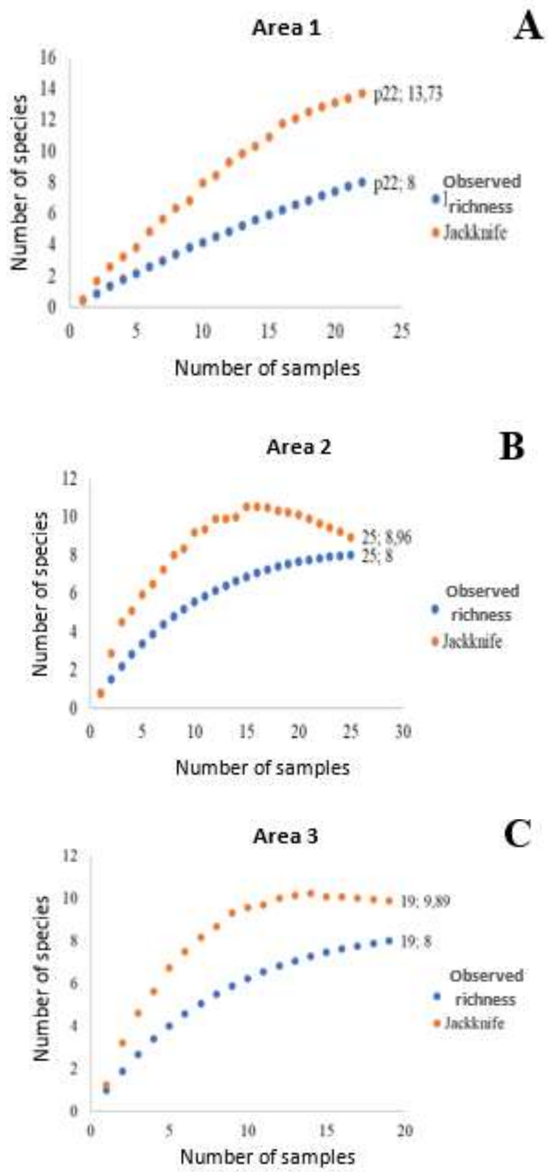

Figure 2 – Species accumulation curves for Scarabaeidae collected in the Inhamum Environmental Protection Area, Caxias, Maranhão, Brazil, in October 2020 and May 2021. (A) Road verge. (B) Cerrado sensu stricto (250 m from the road verge). (C) Riparian forest (500 m from the first Cerrado transect).

Source: Authors (2021).

4. Final remarks

On the basis of the Scarabaeidae population survey conducted in three phytophysiognomies in the Inhamum EPA, the following is concluded: a total of 12 dung beetle species were sampled in two collections in all areas; areas 1 and 2 had the highest abundance of Scarabaeidae; the most frequent, constant, abundant, and dominant species were *Dichotomius nisus* and *Ateuchus* sp.; *Dichotomius* sp. and *Uroxys* were exclusive to Area 1 and *Eurysternus caribaeus* and *Ontherus* sp. to Area 3; area 2 did not have any exclusive species; area 3 had the highest diversity index; soil temperature and rainfall influenced the abundance of Scarabaeidae species; and the richness estimator and accumulation curves revealed that the sampling effort was not sufficient to fully quantify the species; the richness of Scarabaeidae was undersampled in the Inhamum EPA.

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