Nesting Biology of the Solitary Wasp *Pisoxylon amenkei* (Hymenoptera, Crabronidae, Trypoxylini) in a Neotropical Hotspot of Southern Brazil

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Pisoxylon amenkei is a specie of solitary wasp that builds its nests in pre-existing cavities, and has only been recorded nesting in the Araucaria forest, one of the Atlantic forest phytophysiognomies, which is considered a biodiversity hotspot. So far, the only information on the biology of the genus *Pisoxylon* is based on one species. In addition, the genus has many similarities to *Trypoxylon*, showing an increased need for studies on the biology of these species of wasps. In the present study, we brought unpublished information about *P. amenkei* nesting biology and described many aspects of its natural history. It was carried out between August 2017 and August 2019, in a rural area of Guarapuava municipality, Paraná, Brazil. *P. amenkei* nested only in fragmented parts of Araucaria forest, during summer and autumn. It is a multivoltine species, and thus remains in diapause, in the pre-pupa phase during winter and spring. Their nests had an interior design similar to that of *Pisoxylon xanthosoma* and some *Trypoxylon* species, such as *Trypoxylon lactitarse* and *Trypoxylon agamenon*. Moreover, the sex ratio of *P. amenkei* was 1: 1, which can be linked to an equal cost in the production of females and males. Like others species of *Pisoxylon*, *P. amenkei* nests primarily in forest areas. In southern Brazil it nests in fragments of Araucaria forest, which are threatened. Therefore, more efforts are needed for these remaining fragments to be preserved, otherwise our responsibility will be even greater considering the list of extinct species. We can conclude that are necessary further studies focusing on the phylogeny of the group and the use of molecular analyses are needed to clarify the hypothesis of Antropov (1998), that *Pisoxylon* would have to be classified as a subgenus of *Trypoxylon*.

Key words: Hymenoptera, Crabronidae, Diapause, Multivoltine, Conservation.

BACKGROUND

Pisoxylon, described in 1968 by Arnold S. Menke, is a genus of solitary wasp, which builds its nests in pre-existing cavities in forest areas, (Morato and Campos 1994; Iantas et al. 2017; Nether et al. 2019). This genus has three described species: *Pisoxylon roosevelti* Antropov 1998, occurring in Bolivia; *Pisoxylon xanthosoma* Menke, 1968, occurring in Brazil and Peru and *Pisoxylon amenkei* Antropov 1998 occurring only in Brazil, in Paraná and Rio de Janeiro states (Antropov 1998; Amarante 2002). In southern Brazil *P. amenkei* has been recorded as a rare species, occurring with low frequency in trap nests (Iantas et al. 2017; Nether et al. 2019) and nesting only in Araucaria forests (Mixed Ombrophylous Forest) one of phytophysiognomies of the Atlantic forest, and one of the most threatened biomes due to the reduction of its vegetation cover, leaving only isolated fragments (Ribeiro et al. 2011).

Currently the Atlantic forest is among the 35 biodiversity hotspots in the world, and among biomes with priority for conservation, due to the high levels of richness and endemism that it presents, associated with the destruction suffered in the past. Current data indicate that only 28% of its original area remains (Rezende et al. 2018). It is the fifth richest hotspot in endemism (Mittermeier et al. 2004), but many of its species have not yet been sampled or even described. Studies have indicated that its coastal areas have maintained populations of species from the late Quaternary to the present day, making these zones ecological refuges for biodiversity (Carnaval et al. 2009). Despite this, knowledge about many groups of animals, particularly insects, that live in these forests is still incipient (Freitas and Marini-Filho 2011).

Little is known about the wasp nesting biology that occurs in this biome. With the modification of the habitats, the populations of predatory wasps, which nest in branches or tree trunks, have also been altered (Tylianakis et al. 2006) for not finding suitable places to build their nests. Consequently, the rate of predation by these insects has decreased causing changes in the populations of their prey and their natural enemies (Holzschuh et al. 2009). How these three functional guilds remain connected in the ecosystem where they live (LaSalle and Gauld 1993) they are relevant indicators of ecological changes, due to their participation in the three types of interaction (Tscharntke et al. 1998), which makes them bioindicators (Gerlach et al. 2013).

Pisoxylon, as well as several species of the Crabronidae family, uses prey spiders (Buschini et al. 2008; Buschini et al. 2010a; Buschini et al. 2010b; Pitilin et al. 2012; Moura et al. 2019;

Musicante and Salvo 2010) and are parasitized mainly by parasitoid wasps (Nether et al. 2019). These wasps capture a wide range of spider taxa, including orb-weaving spiders (*e.g.*, Araneidae, Tetragnathidae, Nephilidae), araneida sheet web weavers (*e.g.*, Theridiidae), and even several taxa that usually do not use webs to capture prey (*e.g.*, Lycosidae, Salticidae, Oxyopidae, Clubionidae) (Coville and Coville 1980; Camillo and Brescovit 1999; Blackledge et al. 2003, Moura et al. 2019; Buschini et al. 2010a; Buschini et al. 2010b; Pitilin et al. 2012). Knowledge about the biology of this genus is based only on what has been documented for *P. xanthosoma*, with nests containing from one to four cells organized linearly, which are separated by clay partitions (Morato and Campos 1994; Antropov 1998). However, there is no information about their mortality rate, time of development, number of generations and sex ratio, structure and organization of the cocoons and disposition of the sexes in the nests (Antropov 1998).

In addition to the lack of information on the biology of these species, another factor that increases the need for studies on this group is the similarity between the genera *Pisoxylon* and *Trypoxylon* (both from the Trypoxylini tribe), mainly in the way they build their nests, considering only the *Trypoxylon* species that nest in pre-existing cavities (Antropov 1998; Amarante 2002; Coville et al. 1987). Moreover, Menke (1968) considered the compact abdomen of *Pisoxylon* as an autopomorphy, however, with the description of *T. monjuba* Amarante 1995, it was discovered that this species had the first abdomen segment compact, similar to that of *Pisoxylon*, making the relationship between these genders even closer. So much so that according to Antropov (1998), the genus *Pisoxylon* should be included as a subgenre of *Trypoxylon*. Recently, a study by Sann et al. (2018), compiling a comprehensive set of phylogenomic data on the main lineages of Apoid wasps and bee families, confirmed a polyphyly of the Crabronidae family, which reinforces the need for a general review of this group. Although many studies have been carried out on the biological aspects of *Trypoxylon* species (Buschini et al. 2006; Buschini and Wolff 2006; Santoni et al. 2009; Buschini et al. 2008; Fajardo and Buschini 2008; Buschini and Fajardo 2010; Buschini et al. 2010a; Buschini et al. 2010b; Musicante and Salvo 2010; Buschini and Donatti 2012; Moura et al. 2019), there is only one article on the biology of *Pisoxylon* (Morato and Campos 1994). Therefore, more research is needed on the species of this genus, since they help the Systematists to elucidate aspects about the phylogeny of these animals.

In the present study, we brought unpublished information about *P. amenkei* nesting biology and described many aspects of its natural history.


MATERIALS AND METHODS

Study site

This study was carried out between August 2017 and August 2019 in a rural area in the municipality of Guarapuava, Paraná, Brazil. According to the Köppen classification (Kottek et al. 2006), this municipality has a Subtropical Humid Mesothermal climate, with no dry season, severe frosts, an average annual temperature around 22ºC and average annual precipitation of 1961 mm. The months that have the highest temperatures are from September to April, with average temperatures of 23.5°C and the lowest temperatures extends from May to August, with average temperatures of 12.8°C and records of absolute minimums below 0°C (IAPAR, 2014).

We collected in four forest areas and four open grassland areas (where the forest was removed) in two rural areas (Jordão neighborhood and locality of Guabiroba). The forest areas were denominated FJ1 (25°26'58.0"S 51°27'02.1"W), FJ2 (25°26'06.2"S 51°26'34.0"W), FG1 (25°23'56.1"S 51°25'34.4"W) and FG2 (25°23'48.2"S 51°24'49.3"W). The open grassland areas were denominated OJ1 (25°26'50.6"S 51°27'10.9"W), OJ2 (25°25'48.5"S 51°26'50.9"W), OG1 (25°23'43.1"S 51°25'27.7"W) and OG2 (25°24'15.4"S 51°23'44.0"W). All forest fragments used for collection were native and had sizes of FJ1 with 32.9ha, FJ2 with 113ha, FG1 with 256ha and FG2 with 153ha. All these fragments are linked to other nearby fragments which makes their sizes even bigger. In addition, all sampling stations (see the next session) located in the forest were at least 50m away from the edge. All properties had plantations in their vicinity (Fig. 1).

Rural areas FJ1, FJ2, OJ1 and OJ2 were monitored from 2017 to 2019 and areas FG1, FG2, OG1 and OG2 from 2018 to 2019.
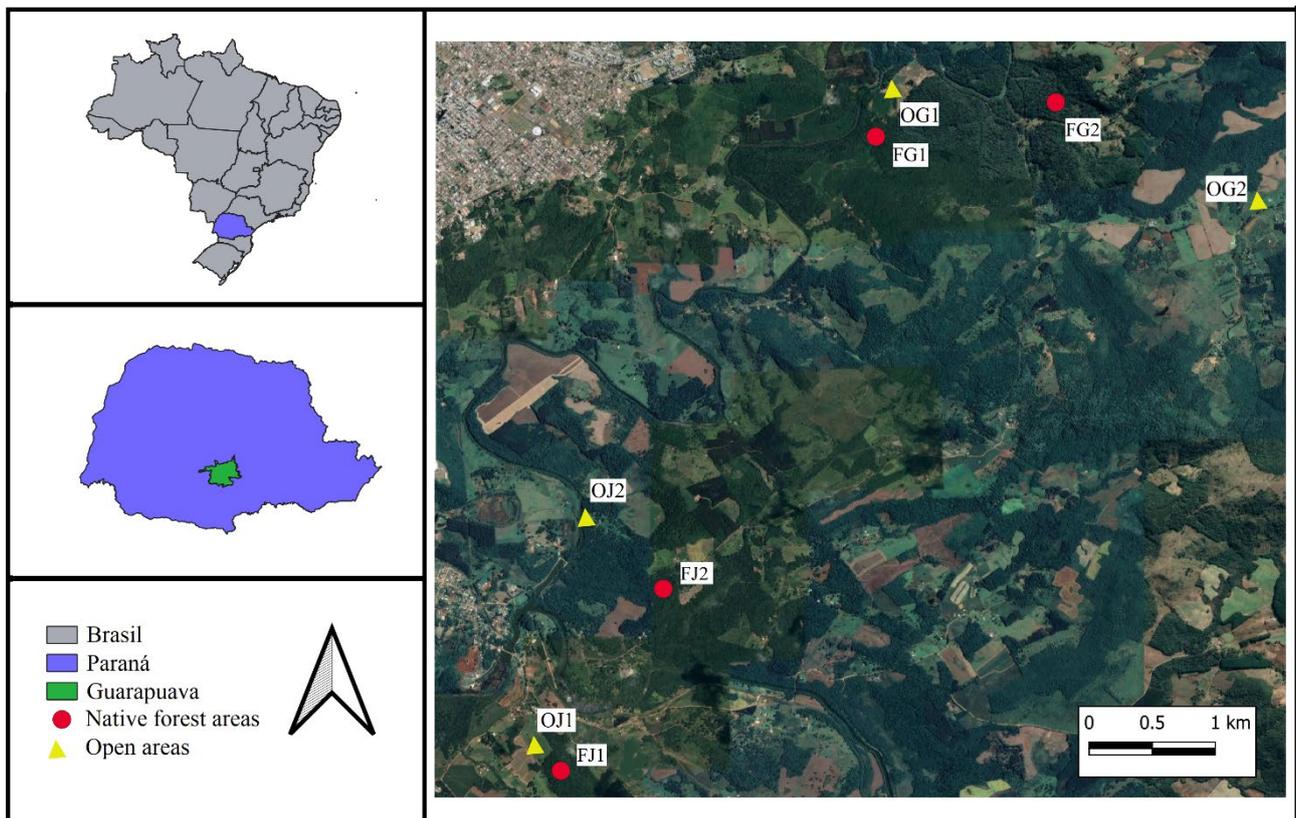
Fig. 1. *P. amenkei* nest collection areas. F: Forest fragment, O: Open grassland, J: Jordão and G: Guabiroba.

Trap nests

We used trap nests to describe the biology of *P. amenkei*, which are pre-existing artificially built cavities. Both nests made out of wood and bamboo. The wooden nests were standardized of 2 × 2 × 12 cm. The cavities measured 0.5, 0.7, 1.0 and 1.3 cm in diameter and were 8 cm deep. Bamboo nests, on the other hand, could not be standardized due to the natural range of sizes, therefore were used those that had a length ranging from 8 to 14 cm, and cavities ranging from 0.4 to 1.5 cm in diameter. Both the wooden and bamboo trap nests were cut longitudinally and joined with adhesive tape so that their interior could be seen.

In each collection area, 6 sampling stations were arranged, which were separated from each other by at least 50 m of distance, totaling 48 sampling stations and 1,536 trap nests. Each sampling station contained two blocks with 16 trap nests each: one block with wooden trap nests and the other with bamboo. The arrangement of trap nests in the blocks was random. They were placed 1.5 m from the ground, on a wooden stake and covered with a roofing tile to prevent the accumulation of rain water.

Collections were carried out every 15 days throughout the 3 years. Trap nests containing wasp or bee nests that were already finished were removed and replaced with a new nest of the same diameter or similar size for bamboo, and taken to the laboratory, where they were placed in

PET (Polyethylene terephthalate) bottles capped with cotton for later emergence and identification of adults. Afterwards, adults were killed under Ethyl acetate, then weighed and tagged. The nests that were identified as belonging to *P. amenkei*, were separated to study their biology. All individuals are deposited in the collection of the Laboratório de Ecologia e Biologia de Vespas e Abelhas da UNICENTRO, Guarapuava, Paraná.

Nests contents

A digital caliper was used to measure brood cells, vestibular and intercalary cells, and the thickness of nest closure. The brood cells were had wasp prey, larvae or cocoons. Vestibular and intercalary cells are the empty cells located immediately after the nest closure, or between two brood cells. Unfortunately, for nests with diameters of 0.7, 1 and 1.3 cm it was possible to measure the length of only one cell, due to the delimitation of each cell not being sufficiently clear.

As the cocoons of *P. amenkei* have a cylindrical shape, their size was calculated by the volume occupied inside the nest, using the formula of the volume of a cylinder: $V = \pi.r^2.h$. Where: V = volume; π (pi) = 3.14; r^2 = radius (half the diameter of the cocoon); h = cocoon length.

The weight of the newly emerged adults was recorded using a Bel Mark precision scale with four decimal places. Using a Leica MZ6 stereomicroscope, the width of their heads (greater distance between the surfaces of the compound eyes in frontal view) and intertergular distance ITD (distance between the inner margins of the tegules) were measured.

The development time of these wasps was calculated as the number of days from the collection of the nests to the emergence of adults in the laboratory. The number of adult generations of this species, was calculated considering the month in started to build their nests, the time that the immature ones took to complete the development, and the month in which the adults stopped nesting entered in diapause.

All data collected were tested for normality by the Shapiro-Wilk test, where everyone has failed and is therefore non-normal data. So, to verify if there was a significant difference between the study variables, the Mann-Whitney non-parametric test was used.

Due to the difficulty of manipulating individuals within the laboratory, the measures used varied in sample number. Only the weight of those individuals that emerged and shortly afterwards were killed under ethyl acetate were used, therefore, individuals that were dead inside nests or inside PET bottles, were not measured. Likewise, measurements of intertergular distance, head width, cocoon volume and cell size were taken only when the object of interest was not damaged (Table 1).

Table 1. Explanation of the sample numbers used

Measures	Adults		Eggs	Larvae	Pupa	Parasitoids	total cells
	74		10	5	12	5	106
	54 alive	20 dead					
Weight (g)	52 - Only individuals who emerged and were killed under ethyl acetate and that could be sexed were weighed.						
Intertegular distance (mm)	71 - of the total number of individuals (living and dead) 71 did not have a damaged thorax and that could be sexed were measured.						
Head width (mm)	70 - from total number of individuals (living and dead) 70 did not have their heads damaged and that could be sexed were measured.						
Cocoon volume (cm³)	66 - of the total number of individuals (living and dead) 66 did not have their cocoons damaged and that the adults could be sexed were measured.						
Cell length (cm)	66 - of the total number of individuals (living and dead) 66 did not have the margins of their cells damaged and that the adults could be sexed were measured.						

Sex ratio and position

The sex ratio was calculated by the ratio between the number of females and the number of males, using the binomial test to verify whether the calculated sex ratio was significantly different from the expected 1: 1 ratio. All statistical analyses were performed using software R, version 3.6.2 (R development core team 2020).

Aiming to check the position of the sexes inside the nest, each cocoon found was removed from its nest and separated into transparent plastic bottles, capped with cotton, containing information such as the number and diameter of the nest, the day it was collected, the collection area and which cell nest it was in. The cocoons stayed in those bottles until the adults emerged.

Mortality and natural enemies

All deaths were counted in five categories: death of eggs, death of larvae, death of pupae, death of adults from unknown causes and death from natural enemies.

RESULTS

During the two years of collection, and a total of 1,536 trap nests available, 59 of them were founded by *P. amenkei*, and 106 cells with individuals were constructed. From total of cells, 74 of them contained adult individuals (54 alive and 20 dead), 10 eggs, 5 larvae, 12 pupae and 5 parasitoids (Table 1). One nest was built in the same trap-nest as *Michantidium* sp., a bee in the Megachilidae family.

All nesting occurred in the forest fragments, which had an average size of 138.7 ha. In addition, the nests were constructed only in the hottest months of the year, from January to May in

2018, entering diapause from May and restarting activities in December of the same year, nesting until June 2019. The greatest frequency nesting took place in January both years (Fig. 2).
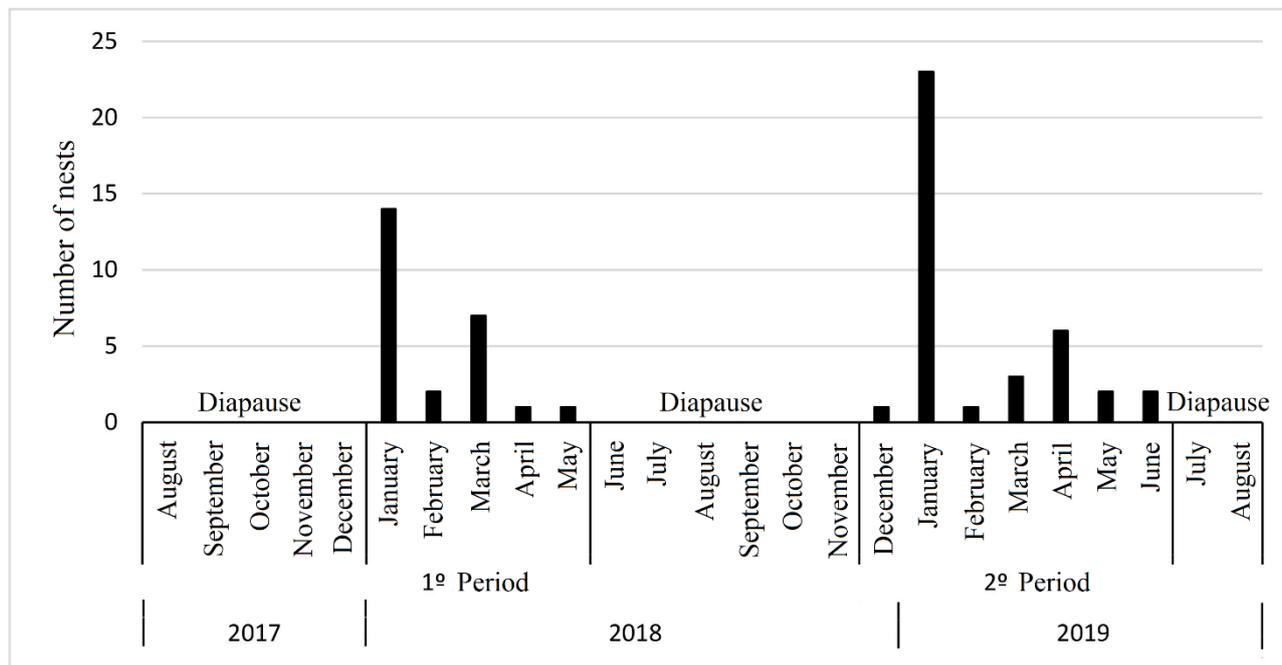
Fig. 2. Number of nests of *P. amenkei* obtained monthly from August 2017 to August 2019.

Nest contents

P. amenkei built their nests preferentially in wooden trap nests, and only one nest was established in a bamboo trap. The most used diameter was 0.5 cm (39 nests), followed by 0.7 (14 nests), 1.3 (4 nests) and 1.0 cm (1 nest). Regardless of the diameter used to nest, the interior design of the nests was similar (Fig. 3). In all nests, the cells and cocoons were arranged linearly. The number of cells ranged from 1 to 7 (Table 2).

The partitions between the cells were built with brown clay, which were convex on the outside and concave on the inside. The average cell size was 1.97 cm. In closure plug, these wasps used organic matter containing resin and vegetable pieces (tiny pieces of leaves and wood), in addition to using clay in the innermost part of the closure plug, providing considerable resistance to the entry of the nests (average thickness of 0.305 cm) (Table 2, Fig. 3). Most nests (50 nests) had vestibular cells soon after this closure, with an average of two vestibular cells per nest. In addition, 21 nests had intercalary cells with an average of 1.4 cells per nest (Fig. 3).

The cocoon of *P. amenkei* has the shape of a cylinder with its concave ends. The posterior end is more tapered than the previous one regarding the entrance of the nest. These cocoons are smooth on the inside and outside and have a light brown and opaque color becoming beige at the ends. All cocoons are covered and fixed to the sides of the nest by silk produced by the larva.

To feed the larvae, this wasp uses spiders immobilized with venom (average of 5 per cell), so that they remain alive for consumption.

Table 2. Measurements of *P. amenkei* trap nests

Diameter	Number of nests	Thickness of closure plug (cm)	Number of cells provisioned	Length of cells (cm)
0.5	39	$\bar{x} = 0.305 \pm 0.106$ md = 0.300	$\bar{x} = 1.769 \pm 0.776$ md = 2	$\bar{x} = 1.973 \pm 0.434$ md = 1.927
0.7	14	$\bar{x} = 0.306 \pm 0.095$ md = 0.311	$\bar{x} = 2 \pm 0.960$ md = 2	2.765*
1	2	$\bar{x} = 0.306 \pm 0.087$ md = 0.291	$\bar{x} = 1.5 \pm 0.707$ md = 1.5	2.100*
1.3	4	$\bar{x} = 0.287 \pm 0.129$ md = 0.274	$\bar{x} = 1.5 \pm 0.577$ md = 1.5	1.800*
TOTAL	59	$\bar{x} = 0.305 \pm 0.106$ md = 0.305	$\bar{x} = 1.830 \pm 0.854$ md = 2	$\bar{x} = 1.972 \pm 0.428$ md = 1.927

$\bar{x}$ indicates the mean with the standard deviation; md indicates the median. * it was possible to obtain the length of just one cell.
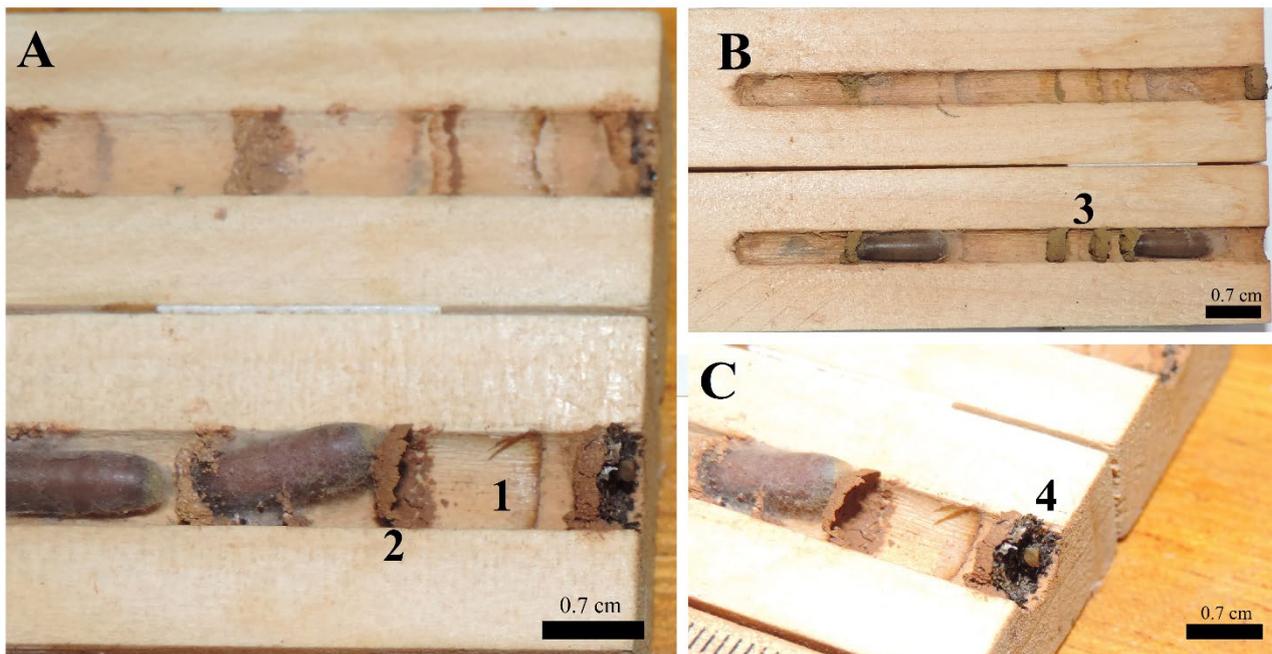
Fig. 3. Nest of *P. amenkei*. A1: a vestibular cell; A2: cell partition; B3: intercalary cells; and C4: closure plug.

Sex ratio and position

P. amenkei have two different times of development times: either they pupated and emerged as adults later in the same season (direct development), or they entered diapause, overwintering and pupating the following spring (delayed development) (Table 3).

Table 3. Development time of *P. amenkei*

	N Sample	Male	Female	Mann-Whitney test (U)	*p*
Direct development (days)	M (5) F (13)	x̄ = 34.2 ± 4.7 md = 33	x̄ = 32 ± 3.7 md = 31	21.5	0.294
Delayed development (days)	M (18) F (17)	x̄ = 246.2 ± 58.9 md = 251	x̄ = 262.5 ± 52.4 md = 287.5	109	0.381

x̄ indicates the mean with the standard deviation; md indicates the median. M: Male; F: Female.

Considering the average time of direct development of the species (37.4 days), *P. amenkei* may have had up to four generations in 2018, and up to six generations in 2019. The first nest collection of *P. amenkei* nests was in January 2018, which means that first generation adults completed their development and nest in February (2018), second generation adults in March (2018), third generation adults in April (2018) and fourth generation adults in May (2018). In addition, this same reasoning, from December 2018 to June 2019, these wasps had up to six generations since they entered diapause later.

The sex ratio of *P. amenkei* was 39 females and 32 males, which did not differ significantly from the expected ratio of 1: 1, considering only adult males and females, not including pupae. Moreover, when we calculate the sex ratio from the two development times separate (direct and delayed development), did not differ significantly too. For this analysis of the sex ratio for the two times of development we used only the individuals from which these times could be measured, being 5 males and 13 females for direct development and 18 males and 17 females for delayed development (Table 4).

Of the total of 51 nests from which the sexes could be identified, the most of them ($n = 43$), were occupied by only one sex, with an average of 1.256±0.539 individuals per nest. In the other eight nests, males and females emerged, all of whom had two individuals. In these nests, with the exception of one, the males were found in the anterior cells and the females in the posterior cells.

Females were significantly larger than males, both in weight and in the intertegular distance and width of head. The cells where the females developed and their cocoon, were also significantly larger than those of males (Table 5, Fig. 4).

Table 4. Binomial test for *P. amenkei* sex ratio

	Males	Females	Probability of success	*p*
Direct development	5	13	0.722	0.096
Delayed development	18	17	0.472	0.868
Total	32*	39*	0.549	0.477

* These values represent both individuals who had their development times measured, as well as those who could not have that time measuring.

Table 5. Adult size and cocoon dimensions of *P. amenkei*

	N Sample	Male	Female	Mann-Whitney test (U)	p
Weight (g)	M (22) F (30)	$\bar{x} = 0.011 \pm 0.005$ md = 0.010	$\bar{x} = 0.023 \pm 0.005$ md = 0.025	49.5	2.141 e^{-07}
Intertegular distance (mm)	M (32) F (39)	$\bar{x} = 1.311 \pm 0.099$ md = 1.344	$\bar{x} = 1.672 \pm 0.137$ md = 1.667	29	5.122 e^{-12}
Head width (mm)	M (32) F (38)	$\bar{x} = 2.214 \pm 0.119$ md = 2.210	$\bar{x} = 2.744 \pm 0.196$ md = 2.812	56	6.436 e^{-11}
Cocoon volume (cm³)	M (30) F (36)	$\bar{x} = 0.089 \pm 0.027$ md = 0.089	$\bar{x} = 0.174 \pm 0.059$ md = 0.179	91	7.661 e^{-09}
Cell length (cm)	M (30) F (36)	$\bar{x} = 1.813 \pm 0.341$ md = 1.812	$\bar{x} = 2.106 \pm 0.451$ md = 2.079	333.5	0.008

$\bar{x}$ indicates the mean with the standard deviation; md indicates the median. M: Males; F: Females.

Fig. 4. *P. amenkei* female. General body coloration dark brown, in frontal view, head somewhat widened dorsally. Median clypeal lobe convex in the middle and strongly protruding, trapeziform; laterally with frontal depression and bent frontally along anterior margin (Antropov 1998).

Mortality and natural enemies

From the total of 106 cells that contained individuals supplied by nests of *P. amenkei*, there was mortality in 52 of them (49.05%). From these, 10 deaths were eggs (~20%), most likely due to dehydrated spider prey; 5 cells with dead larvae (~10%); 12 cells with dead pupae (~24%); 19 cells with adults died from unknown causes (~36%). *Messatoporus* sp. and *Photochryptus* sp., parasitoids of the Ichneumonidae family, caused mortality in four cells (~8%) and one cell (~2%).

DISCUSSION

This study was developed in a mosaic landscape composed by Araucaria forest fragments, grassland and monocultures. *P. amenkei* nested only in forest fragments which are characterized by the presence of *Araucaria angustifolia* (Bertolini) (Coniferae; Araucariaceae) that has been classified as vulnerable according to IUCN standards (Hilton-Taylor 2000) and is protected by law (National Forest Code 4771/65), however the conservation status of this forest, itself, is critical (Fonseca et al. 2009). In view of this scenario, we believe that species such as *P. amenkei*, which have been classified as rare and that nest only in these forests (Nether et al. 2019; Iantas et al. 2017), are at serious risk of becoming extinct.

It is possible that one of the factors that influence the distribution of *P. amenkei* in Araucaria forest, in addition to the abiotic conditions, is the availability of spiders and holes to construct its nests, which will undoubtedly affect the distribution of its parasitoids. For example, according to Araújo et al. (2018), that study *Trypoxylon lactitarse* (Saussure, 1867), and Buschini and Fajardo (2010) studying *Trypoxylon agamenon* Richards, 1934, which also nested only in these forest fragments and using pre-existing cavities, and spiders to provision, native forests have a microenvironment with lower temperatures and luminosities, and higher relative air humidity (Buschini 2006; Buschini and Wolff 2006; Buschini and Woiski 2008) in addition to presenting a higher density of prey, or even offering more cavities for nests (Buschini et al. 2006; Buschini et al. 2008; Morato and Martins 2006).

The vegetation structure of a given habitat is generally considered an important factor for spider diversity (Baldissera et al. 2004; Corcuera et al. 2008). The three-dimensional structure generated by plants on a given forest can have a strong influence on the number and variety of spiders that can live on that environment, be they web spinners or hunters (Rodrigues and Mendonça 2012), although anthropogenic changes in the ecosystem such as urbanization and habitat fragmentation in particular (Miyashita et al. 1998, Bolger et al. 2000) has been having a great influence on spider communities in general (Whitehouse et al. 2002).

We are losing many species of arthropods that are vulnerable to habitat fragmentation. The study by Sanchez-Bayo and Wyckhuys (2019) revealed that the extinction rates of the world's insect species in the coming decades could reach 40%, with Hymenoptera being one of the most affected taxa in terrestrial ecosystems. According to these authors, this decline may occur both with specialist species, which occupy particular ecological niches, and many common and generalist species. On parasitoids, the impact of habitat fragmentation is further exacerbated by the fact that they often occur at low densities, in populations that are therefore more likely to be vulnerable to changes (Kruess and Tscharntke 2000; Ewers and Didham 2006). Perhaps this explains the fact that our study recorded for the first time the presence of parasitoids in the nests of *P. amenkei* in southern Brazil. *Messatoporus* and *Photochryptus*, of Ichineumonidae family, were the ones that

emerged from its nest and in low abundance. According to Audusseau et al. (2020), the persistence of the parasitoid population at a site is therefore the result of the interplay between local habitat suitability, species' capacity to disperse between patches and the distribution in time and space of its potential hosts in the landscape.

The interior design of wasp nests can also influence the rate of parasitism (Krombein 1967) by parasitoids such as Ichneumonidae, which has been seen introducing its ovipositor through the closure plug of the *Trypoxylon* nests (Buschini personal information).In addition to the clay in closure plug, *P. amenkei* uses an outer layer of dark and resistant organic matter, which is mixed with vegetable resin, beyond the presence of intercalary and vestibular cells that hinder parasitism by natural enemies (Assis et al. 1997). Their nests were very similar to the nests of *Trypoxylon* species such as *T. opacum*, *T. agamenon* and *T. lactitarse*, with linear arrangement of the cells, partitions between them built out of mud, the presence of vestibular and interlayer cells and provision of cells with spiders (Buschini and Wolff 2006; Buschini and Fajardo 2010; Buschini et al. 2006). What differentiates them is the shape and color of the cocoons, and the closing of the nests (Buschini and Wolff 2006; Buschini 2007; Buschini et al. 2010b).

The disposition of the sexes in *P. amenkei* nests also differs from those of the *Trypoxylon* species that nest in pre-existing cavities. In most *P. amenkei* nests, females and males were raised in separate nests. In shared nests, females were raised in the innermost cells and males in the outermost cells. Both in *T. lactitarse* (Buschini et al. 2006), *T. opacum* (Buschini and Wolff 2006) and *T. agamemnon* (Buschini and Fajardo 2010), the position of the sexes was random and they were laid together, regardless of the diameter of the cavity used for nesting. The position of the sexes of *P. amenkei* was similar to what was registered for *P. xanthosoma*, who also oviposited males and females in separate nests, and in those with shared sexes, in two of them the females emerged from the innermost cells (Morato and Campo 1994). Its sex ratio was 1: 1 which means that the cost of producing males and females is equal. According to Fisher (1930), equal investment in sons and daughters, in most species, is the result of frequency dependence. If the cost of producing sons and daughters is equal, this investment also translates into equal numbers of males and females (Fisher 1930; Seger 1983; Buschini 2007). However, considering that *P. amenkei* females are larger than males, it can be observed that although the sex ratio was 1: 1, parental investment was higher in female production (Buschini 2007).

CONCLUSIONS

Results like those here are part of the so-called basic research, which is a fundamental part of science. These types of research can be very important because they can present results that are often unexpected, thus generating new research fields (Courchamp et al. 2015). In addition, basic research is the basis for future applied research, which are those that have more restricted objectives, such as species conservation and Systematics (Courchamp et al. 2015; Freitas et al. 2020).

We can conclude that *P. amenkei* presented the interior design of nests similar to the *Trypoxylon* species that nesting in cavities, but differed in relation to the shape and color of the cocoons, the closing of the nests and the disposition of the sexes in the nests. Like others species of *Pisoxylon*, *P. amenkei* nests primarily in forest areas. In southern Brazil it nests in fragments of Araucaria forest, which are threatened. Therefore, more efforts are needed for these remaining fragments to be preserved, otherwise our responsibility will be even greater considering the list of extinct species. Therefore, further studies focusing on the phylogeny of the group and the use of molecular analyses are needed to clarify the hypothesis of Antropov (1998), that *Pisoxylon* would have to be classified as a subgenus of *Trypoxylon*.

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REFERENCES

Amarante STP. 1995. *Trypoxylon* (Trypoxylon) *mojuba*, sp. n., an inusual Trypoxylini from South America (Hymenoptera, Sphecidae). Revista Brasileira de Entomologia (Brazil) **39:**157–160.

Amarante STP. 2002. Synonymic catalog of the Neotropical Crabronidae and Sphecidae (Hymenoptera: Apoidea). Arquivos de Zoologia **37:**1–139.

Antropov AV. 1998. Digger wasps of the genus *Pisoxylon* (Hymenoptera, Sphecidae, Trypoxylini). Entomol Rev **78:**761–767.

Araújo GJ, Storck-Tonon D, Izzo TJ. 2018. Is Planting Trees Enough? The Effect of Different Types of Reforestation on the Offspring of *Trypoxylon* (Trypargilum) *lactitarse* (Hymenoptera: Crabronidae) in the Southern Amazon. Neotrop Entomol **48:**572–582. doi:10.1007/s13744-019-00682-9.

Assis JMF, Camillo E. 1997. Diversidade, sazonalidade e aspectos biológicos de vespas solitárias (Hymenoptera: Sphecidae: Vespidae) em ninhos armadilhas na Região de Ituiutaba, MG. Anais da Sociedade Entomológica do Brasil **26:**335–347. doi:10.1590/S0301-80591997000200016.

Audusseau H, Baudrin G, Shaw MR, Keehnen NL, Schmucki R, Dupont L. 2020. Ecology and genetic structure of the parasitoid *Phobocampe confusa* (Hymenoptera: Ichneumonidae) in relation to its hosts, *Aglais* Species (Lepidoptera: Nymphalidae). Insects **11:**478. doi:10.3390/insects11080478.

Baldissera R, Ganade G, Fontoura SB. 2004. Web spider community response along an edge between pasture and Araucaria Forest. Biol Conser **118:**403–409. doi:10.1016/j.biocon.2003.09.017.

Blackledge TA, Binford GJ, Gillespie RG. 2003. Resource use within a community of Hawaiian spiders (Araneae: Tetragnathidae). *In*: Annales Zoologici Fennici (pp. 293–303). Finnish Zoological and Botanical Publishing Board.

Bolger DT, Suarez AV, Crooks KR, Morrison SA, Case TJ. 2000. Arthropods in urban habitat fragments in southern California: area, age, and edge effects. Ecol Appl **10:**1230–1248. doi:10.1890/1051-0761(2000)010[1230:AIUHFI]2.0.CO;2.

Buschini MLT, Borba NA, Brescovit AD. 2008. Patterns of prey selection of *Trypoxylon* (Trypargilum) *lactitarse* Saussure (Hymenoptera: Crabronidae) in southern Brazil. Braz J Biol **68:**519–528. doi:10.1590/S1519-69842008000300008.

Buschini MLT, Borba NA, Brescovit AD. 2010b. Prey selection in the trap-nesting wasp *Trypoxylon* (Trypargilum) *opacum* Brèthes (Hymenprtera; Crabronidae). Braz J Biol **70:**529–536. doi:10.1590/S1519-69842010000300009.

Buschini MLT, Donatti AJ. 2012. Nesting behavior of *Trypoxylon* (Trypargilum) *agamemnom* Richards (Hymenoptera: Crabronidae). Braz J Biol **72:**353–362. doi:10.1590/S1519-69842012000200017.

Buschini MLT, Fajardo S. 2010. Biology of the solitary wasp *Trypoxylon* (Trypargilum) *agamemnon* Richards 1934 (Hymenoptera: Crabronidae) in trap-nests. Acta Zoologica **91:**426–432. doi:10.1111/j.1463-6395.2009.00429.x.

Buschini MLT, Niesing F, Wolff LL. 2006. Nesting biology of *Trypoxylon* (Trypargilum) *lactitarse* Saussure (Hymenoptera, Crabronidae) in trap-nests in southern Brazil. Braz J Biol **66:**919–929. doi:10.1590/S1519-69842006000500018.

Buschini MLT, Woiski TD. 2008. Alpha–beta diversity in trap-nesting wasps (Hymenoptera: Aculeata) in Southern Brazil. Acta Zoologica **89:**351–358. doi:10.1111/j.1463-6395.2008.00325.x.

Buschini MLT, Wolff LL. 2006. Notes on the biology of *Trypoxylon* (Trypargilum) *opacum* Brèthes (Hymenoptera; Crabronidae) in southern Brazil. Braz J Biol **66:**907–917. Doi:10.1590/S1519-69842006000500017.

Buschini MLT. 2007. Life-history and sex allocation in *Trypoxylon* (syn. Trypargilum) *lactitarse* (Hymenoptera; Crabronidae). J Zool Syst Evol Res **45:**206–213. doi:10.1111/j.1439-0469.2006.00405.x.

Buschini MTL, Caldas TR, Borba NA, Brescovit AD. 2010a. Spiders used as prey by the hunting wasp *Trypoxylon* (Trypargilum) *agamemnon* Richards (Hymenoptera: Crabronidae). Zool Stud **49:**169–175.

Buschini MTL. 2006. Species diversity and community structure in trap-nesting bees in Southern Brazil. Apidologie **37:**58-66. doi:10.1051/apido:2005059.

Camillo E, Brescovit AD. 1999. Spiders (Araneae) captured by *Trypoxylon* (Trypargilum) *lactitarse* (Hymenoptera: Sphecidae) in southeastern Brazil. Rev Biol Trop **47:**151–162.

Carnaval AC, Hickerson MJ, Haddad CFB, Rodrigues MT, Moritz C. 2009. Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science **323:**785–789. doi:10.1126/science.1166955.

Corcuera P, Jiménez ML, Valverde PL. 2008. Does the microarchitecture of Mexican dry forest foliage influence spider distribution? J Arachnol **36:**552–556. doi:10.1636/T05-20.1.

Courchamp F, Dunne JA, Le Maho Y, May RM, Thébaud C, Hochberg ME. 2015. Fundamental ecology is fundamental. Trends Ecol Evol **30:**9–16. doi:10.1016/j.tree.2014.11.005.

Coville RE, Coville PL. 1980. Nesting biology and male behavior of *Trypoxylon* (Trypargilum) *tenoctitlan* in Costa Rica (Hymenoptera: Sphecidae). Ann Entomol Soc Am **73:**110–119. doi:10.1093/aesa/73.1.110.

Coville RE. 1987. Spider-Hunting Sphecid Wasps. *In*: Nentwig W. (eds) Ecophysiology of Spiders. Springer, Berlin, Heidelberg. doi:10.1007/978-3-642-71552-5_23.

Ewers RM, Didham RK. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biol Rev **81:**117–142. doi:10.1017/S1464793105006949.

Fajardo S, Buschini MLT. 2008. Aspectos biológicos e sazonalidade de *Trypoxylon* (Trypargilum) *agamemnon* RICHARDS 1934 (Hymenoptera: Crabronidae) em ninhos armadilha no Parque Municipal das Araucárias, Guarapuava–PR. Unicentro-Revista Eletrônica Lato Sensu **4:**19. doi:10.1590/S0301-80591999000200008.

Fisher RA. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.

Fonseca CR, Souza AF, Leal-Zanchet AM, Dutra TL, Backes A, Ganade G. 2009. Floresta com Araucária: Ecologia, Conservação e Desenvolvimento Sustentável. Holos, Ribeirão Preto.

Freitas AVL, Marini-Filho OJ. 2011. Plano de ação Nacional para conservação dos Lepidópteros ameaçados de extinção. ICMBio, Brasília, pp.15–72. http://www.icmbio.gov.br/portal/images/stories/docs-plano-de-acao/pan-lepidoptera/livro_lepidopteras_web2.pdf. Accessed 19 Feb. 2020.

Freitas AVL, Rosa AHB, Nobre CEB, Melo DHA, Mota LL, Silva-Brandao KL, Machado PA, Carreira JYO. 2020. Immature Stages, Natural History, Systematics and Conservation of an Endangered Neotropical Butterfly: the Case of *Scada karschina delicata* (Nymphalidae: Ithomiini). Neotrop Entomol **49:**685–695. doi:10.1007/s13744-020-00797-4.

Gerlach J, Samways M, Pryke J. 2013. Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. J Insect Conser **17:**831–850. doi:10.1007/s10841-013-9565-9.

Hilton-Taylor C. 2000. IUCN red list of threatened species. IUCN, Gland.

Holzschuh A, Steffan-Dewenter I, Tscharntke T. 2009. Grass strip corridors in agricultural landscapes enhance nest site colonisation by solitary wasps. Ecol Appl **19:**123–132. doi:10.1890/08-0384.1.

Iantas J, Gruchowski Woitowicz FC, Buschini MLT. 2017. Habitat modification and alpha-beta diversity in trap nesting bees and wasps (Hymenoptera: Aculeata) in southern Brazil. Trop Zool **30:**83–96. doi:10.1080/03946975.2017.1301628.

IAPAR Instituto Agronômico do Paraná. 2014. Londrina. http://www.iapar.br/. Accessed Feb. 2020.

Kottek M, Grieser J, Beck C, Rudolf B, Rubel F. 2006. World map of the Köppen-Geiger climate classification updated. Meteorol Z **15:**259–263. doi:10.1127/0941-2948/2006/0130.

Krombein KV. 1967. Trap-nesting wasps and bees: Life history, nests, and associates. Washington, DC: Smithsonian Press.

Kruess A, Tscharntke T. 2000. Species richness and parasitism in a fragmented landscape: Experiments and field studies with insects on *Vicia sepium*. Oecologia **122:**129–137. doi:10.1007/PL00008829.

Lasalle J, Gauld ID. 1993. Hymenoptera: their diversity, and their impact on the diversity of other organism. In: Lassale and Gauld, I. D. (eds). Hymenoptera and Biodiversity, CAB International, UK, Wallinford, 1993 pp. 1–26.

Menke AS. 1968. New Genera and Species of Wasps of the Tribe Sypoxylonini from the Neotropical Region (Hymenoptèra, Sphecidae, Larrinae), Los Angeles Co. Mus Contrib Sci **135:**1–9.

Mittermeier RA, Gil PR, Hoffman M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, Fonseca GAB. 2004. Hotspots revisited: earth's biologically richest and most endangered terrestrial ecoregions. Conservation International p. 392.

Miyashita T, Shinkai A, Chida T. 1998. The effect of forest fragmentation on web spider communities in urban areas. Biol Conser **86:**357–364. doi:10.1016/S0006-3207(98)00025-1.

Morato EF, Campos OLA. 1994. Aspectos da biologia de *Pisoxylon xanthosoma* na amazônia central (Hymenoptera, Sphecidae, Larrinae, Trypoxylonini). Revista Brasileira Entomol **38:**585–594.

Morato EF, Martins RP. 2006. An overview of proximate factors affecting the nesting behavior of solitary wasps and bees (Hymenoptera: Aculeata) in preexisting cavities in wood. Neotrop Entomol **35:**285–298. doi:10.1590/S1519-566X2006000300001.

Moura RR, Pitilin RB, Buschini MLT. 2019. Specialisation in prey capture drives coexistence among sympatric spider-hunting wasps. Ecol Entomol **44:**639–647. doi:10.1111/een.12743.

Musicante ML, Salvo A. 2010. Nesting biology of four species of *Trypoxylon* (Trypargilum) (Hymenoptera: Crabronidae) in Chaco Serrano woodland, Central Argentina. Rev Biol Trop **58:**1177–1188.

Nether MC, Dudek J, Buschini MLT. 2019. Trophic interaction and diversity of cavity-nesting bees and wasps (Hymenoptera: Aculeata) in Atlantic forest fragments and in adjacent matrices. Apidologie **50:**104–115. doi:10.1007/s13592-018-0623-x.

Pitilin RB, Araújo MS, Buschini ML. 2012. Individual specialization in the hunting-wasp *Trypoxylon* (Trypargilum) *agamemnon* Richards (Hymenoptera: Crabronidae). Zool Stud **51:**655–662.

R Development Core Team R. 2020. R: A language and environment for statistical computing.

Rezende CL, Fraga JS, Sessa JC, De Souza GVP, Assad ED, Scarano FR. 2018.Land use policy as a driver for climate change adaptation: a case in the domainof the Brazilian Atlantic forest. Land Use Policy **72:**563–569. doi:10.1016/j.landusepol.2018.01.027.

Ribeiro MC, Martensen AC, Metzger JP, Tabarelli M, Scarano F, Fortin MJ. 2011. The Brazilian Atlantic Forest: a shrinking biodiversity hotspot. Biodiversity hotspots. Springer pp. 405–434.

Rodrigues ENL, Mendonça MS JR. 2012. Spider guilds in the tree-shrub strata of riparian forests in southern Brazil. J Arachnol **40:**39–47. doi:10.1636/P10-105.1.

Sánchez-Bayo F, Wyckhuys KA. 2019. Worldwide decline of the entomofauna: A review of its drivers. Biol Conser **232:**8–27. doi:10.1016/j.biocon.2019.01.020.

Sann M, Niehuis O, Peters RS, Mayer C, Kozlov A, Podsiadlowski L, Bank S, Meusemann K, Misof B, Bleidorn C, Ohl M. 2018. Phylogenomic analysis of Apoidea sheds new light on the sister group of bees. BMC Evol Biol **18:**1–15. doi:10.1186/s12862-018-1155-8.

Santoni MM, Brescovit AD, Del Lama MA. 2009. Ocupação diferencial do habitat por vespas do gênero *Trypoxylon* (Trypargilum) Latreille (Hymenoptera, Crabronidae). Rev Bras Entomol **53:**107–114. http://dx.doi.org/10.1590/S0085-56262009000100024.

Seger J. 1983. Partial bivoltinism may cause alternating sex-ratio biases that favour eusociality. Nature **301:**59–62. doi:10.1038/301059a0.

Tscharntke T, Gathmann A, Steffan-Dewenter I. 1998. Bioindication using trap-nests bees and wasps and their natural enemies: community structure and interactions. J Appl Ecol **35:**708–719. doi:10.1046/j.1365-2664.1998.355343.x.

Tylianakis JM, Tscharntke T, Klein AM. 2006. Diversity, ecosystem function, and stability of parasitoid host interactions across a tropical habitat gradient. Ecology **87:**3047–3057. doi:10.1890/0012-9658(2006)87[3047:DEFASO]2.0.CO;2.

Whitehouse MEA, Shochat E, Shachak M, Lubin Y. 2002. The influence of scale and patchiness on spider diversity in a semi-arid environment. Ecography **25:**395–404. doi:10.1034/j.1600-0587.2002.250402.x.