



## RESEARCH ARTICLE - BEES

## Pollen Niche from *Tetrapedia diversipes* Klug (Hymenoptera: Apidae: Tetrapediini) in a Brazilian Semi-deciduous Lowland Forest

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### Abstract

*Tetrapedia diversipes* Klug (Apidae) is a solitary bee with wide geographical distribution. Females of this species usually nest in pre-existing cavities and although their biology is relatively well known, studies on their trophic niche are still scarce. Thus, the present study aimed to identify the plant species used in the diet of immature *T. diversipes* through the analysis of residual pollen material. The nests used in the study were obtained through the use of trap nests during the period between December 2014 and October 2015, distributed in an area of coastal forest in northeastern Brazil. For the study of the trophic niche, samples of post-emergence residue (feces) from cells of offspring of males ( $n = 31$ ) and females ( $n = 66$ ) of *T. diversipes* were analyzed. In total, 29 pollen types were identified in the feeding of *T. diversipes*, being of the pollen types identified, only seven were not used by both sexes. Although a significant difference was found in the amplitude in the trophic niche between the sexes, there was a high overlap for Horn-Morisita index ( $CH = 0.989$ ), with no significant difference being found in the frequency distribution of male and female food items ( $D = 0.214$ ;  $p = 0.341$ ). In this study we concluded that the diet of *T. diversipes* in coastal forest is polylectic, similarly to the pattern found in other studies of pollen resources in different vegetation in Brazil, with a predominance of pollen from the families Euphorbiaceae, Asteraceae and Onagraceae.

### Introduction

Most species of bees (Insecta: Hymenoptera: Apoidea) have solitary habits and express a close relationship with the flowers to obtain resources for nest provisioning and their own nutrition (Batra, 1984; Barth, 1991; Michener, 2007). Depending on the species of solitary bee, it can use different materials to build their nest such as resin, sand, sawdust, resin with pieces of wood or leaves and generally use nectar and pollen to feed their descendants (Alves-dos-Santos et al., 2002; Pinheiro et al., 2014). However, some groups of bees have specific interactions with floral oil and also use this material as part of their larvae diet and as an auxiliary resource in the construction of their nests (Simpson & Neff, 1981; Buchmann, 1987; Michener, 2007; Pinheiro et al., 2014; Neff & Simpson, 2017; Lourenço et al., 2019).

Nectar is the main energy source while pollen is the protein source of the bee diets (Souza et al., 2004). Also, pollen grains have a range of compounds such as lipids and vitamins that are essential for the proper offspring development (Souza et al., 2004; Marchini et al., 2006; Kriesell et al., 2017; Ruedenauer et al., 2020). There are several studies on the importance of pollen in bee nutrition (Crailsheim, 1990; Funari et al., 2003; Gregory, 2006; Pinto et al., 2019), but taxonomic studies on the pollen grains and the plasticity of the trophic niche of certain bee species are necessary for a better understanding of this bee-plant relationship (Dórea et al., 2009; Dórea et al., 2010; Menezes et al., 2012; Neves et al., 2014; Rocha-Filho et al., 2018; Santos et al., 2020).

The genus *Tetrapedia* Klug has Neotropical distribution and is composed of 28 valid species, with *T. diversipes* being the species with the largest range of occurrence records,

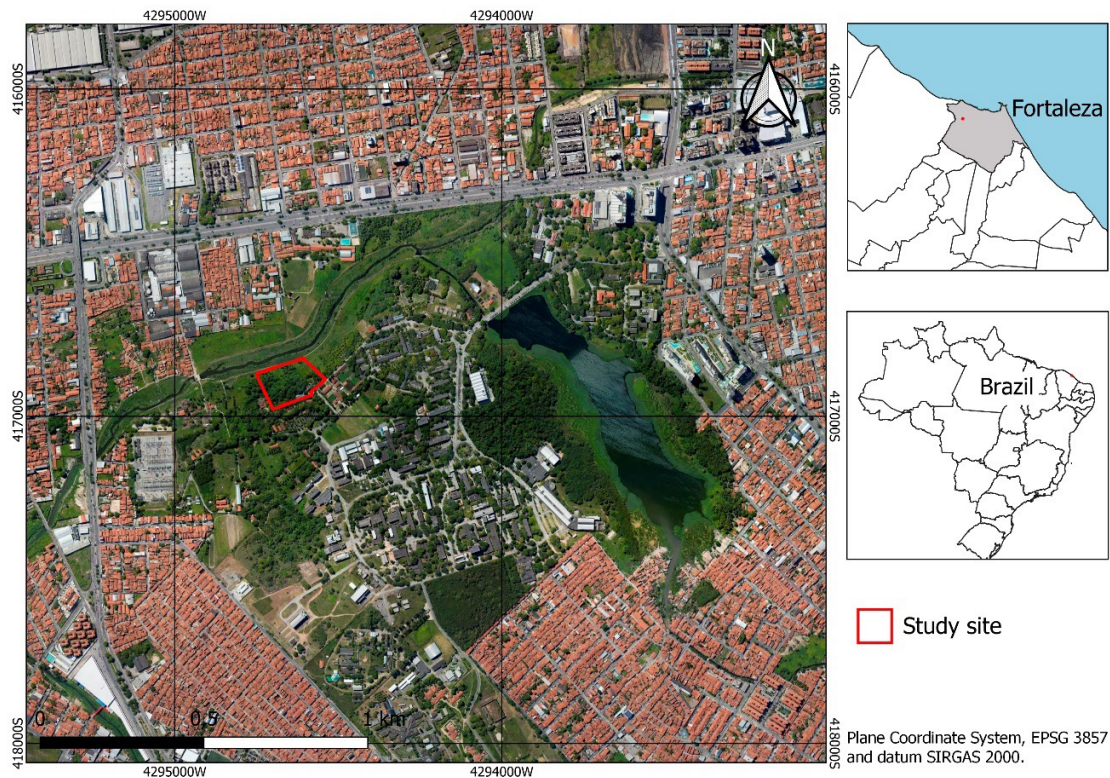


from San José (Costa Rica) to Misiones (Argentina) (Moure, 2012). Although the biology of *T. diversipes* is relatively well-known (Camillo, 2005; Menezes et al., 2012; Rocha-Filho & Garófalo, 2015; Cavalcante et al., 2019), studies on its trophic niche are still scarce and restricted to the Atlantic forest and coffee agrosystems (Menezes et al., 2012; Neves et al., 2014; Rocha-Filho & Garófalo, 2015; Rocha-Filho et al., 2018) thus, it is necessary to obtain a greater knowledge about which plants provide the floral resources used by this bee when supplying its nest. In this context, this study aimed to identify the plant species used in the immature diet of *T. diversipes*, through the analysis of the pos-emergence residue in their nesting cells and to verify whether the bee makes different collections among the plant species used in the individually supply cells in which males and females have developed. We intend to increase knowledge about the trophic niche of these bees and obtain information that may provide subsidies for the conservation of these native populations in Semi-deciduous Lowland Forest.

## Material and methods

### Study area and trap nest

The study was carried out in the Bee Unit of Prof. Prisco Bezerra Campus of the Federal University of Ceará, popularly known as Campus do Pici (3°34'16" S and 38°34' 42" W), located in the city of Fortaleza, State of Ceará, Brazil (Fig 1). This Campus is composed of an extensive wooded area with native and exotic species forming the current landscape and containing a small fragment of vegetation (8 ha) of Semi-deciduous Lowland Forest (Mata de Tabuleiro, IBGE 2012). This forest is located inside an area recognized as an Area of Relevant Ecological Interest (ARIE of "Matinha do Pici" with 42.62 ha) according to Municipal Law 10.463, of March 31, 2016, which provides for the creation of the ARIE (Vasconcelos et al., 2019). The climate is Aw' (Köppen 1948) with an average annual rainfall of 1,448 mm and an average annual temperature of 26.3 °C (Climate-Data, 2020).

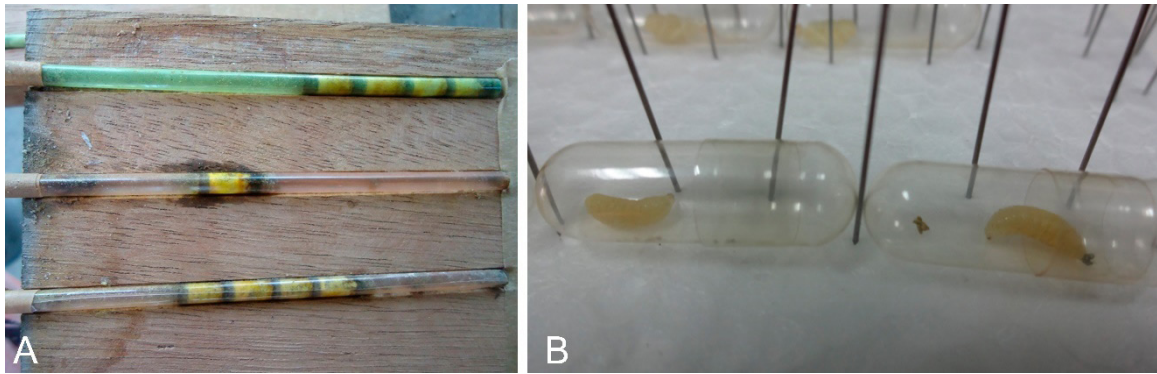


**Fig 1.** Geographic location of the study area in the Bee Unit at the Campus do Pici/ Federal University of Ceara, Fortaleza-CE, Brazil.

The nests were obtained by the provision of trap nests (TN) made of black cardboard of cylindrical shape, 5.0 mm in diameter and 12 cm in length, and transparent plastic straws (4.5 mm x 12 cm). Both TNs had a closed end with a wax blend of *Melipona subnitida* Ducke, 1910 and *Scaptotrigona* sp. nov. (Oliveira *in litt.*). These TNs were inserted into nine wooden blocks with 548 pre-existing cavities, the blocks were located 90 cm above ground level. The TNs of plastic straw were installed in only one of the nine blocks used in the study, for this, 36 TNs of cardboard were replaced by transparent plastic straws (Fig 2A), to facilitate the observation inside the nest.

Paper TNs were installed in September 2014, and after *T. diversipes* females began to nest, they were monitored daily for nine months (December/2014 to September/2015), while plastic straw TNs were evaluated four months (June to October/2015). The nests found on cardboard were transferred to the laboratory and kept within a Biochemical Oxygen Demand (B.O.D.) at 27 °C until the emergence of adults. Nests found on the plastic straws were also placed in B.O.D., but only until the larvae defecated. At this time, the immatures were transferred to artificial cells made from biodegradable capsules (Fig 2B) to complete their development. A couple





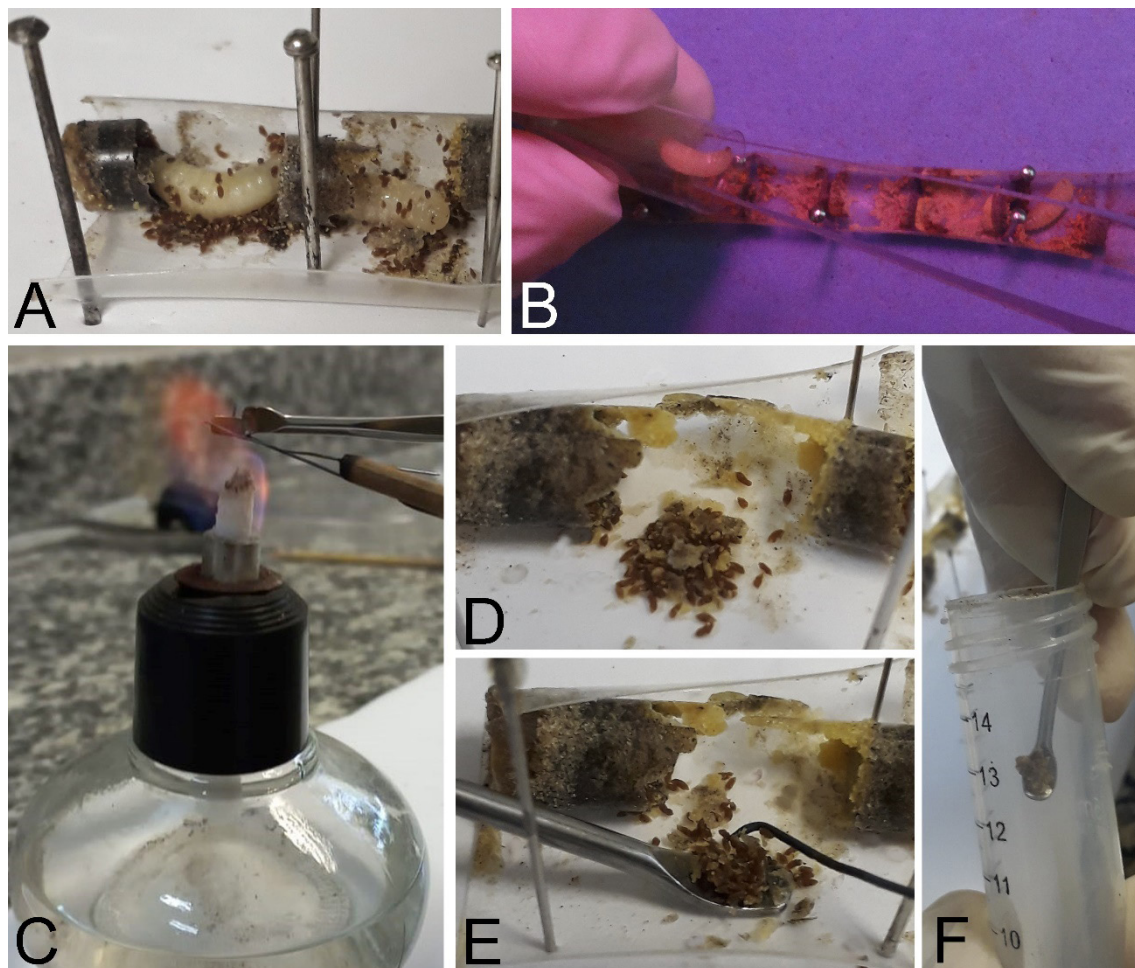
**Fig 2.** Trap nests and immatures of *Tetrapedia diversipes* Klug in the Bee Unit, Campus do Pici/Federal University of Ceara, Fortaleza-CE, Brazil: Trap nests used to facilitate the observation of nest construction by bees (A). Biodegradable capsules used to complete the immature development of bees after opening the nests (B).

from each nest was sacrificed and later deposited in the bee collection of the Laboratório de Abelhas of the Departamento de Zootecnia, UFC.

#### *Analysis of the post-emergence residue*

After the emergence of adult individuals, TNs (n = 49) were opened to collect the residual feces present inside the cells (n = 182). The collected material was placed in tubes with

2 ml of alcohol 70% and processed by the acetolysis method (Erdtman, 1960), following the protocol established by Silva et al. (2014). Soon after the acetolysis process, the pollen was placed in glycerin 50% for at least 24h. Slide duplicates were prepared for each sample, with pollinic material in the Kisser glycerin gelatin (Kisser, 1935). The slides were sealed with colorless enamel and individually labeled with information about nest origin.



**Fig 3.** Procedure for collecting feces samples inside the cells of immatures of *Tetrapedia diversipes* Klug in the Bee Unit at the Campus do Pici/Federal University of Ceara, Fortaleza-CE, Brazil: Open nest containing larvae with feces (A). Transfers from immatures to capsules (B). Hygienization of the material before starting each collection (C). Feces moistened with 70% alcohol (D). Feces collection with spatula (E). Transfer of feces to the falcon tube (F).

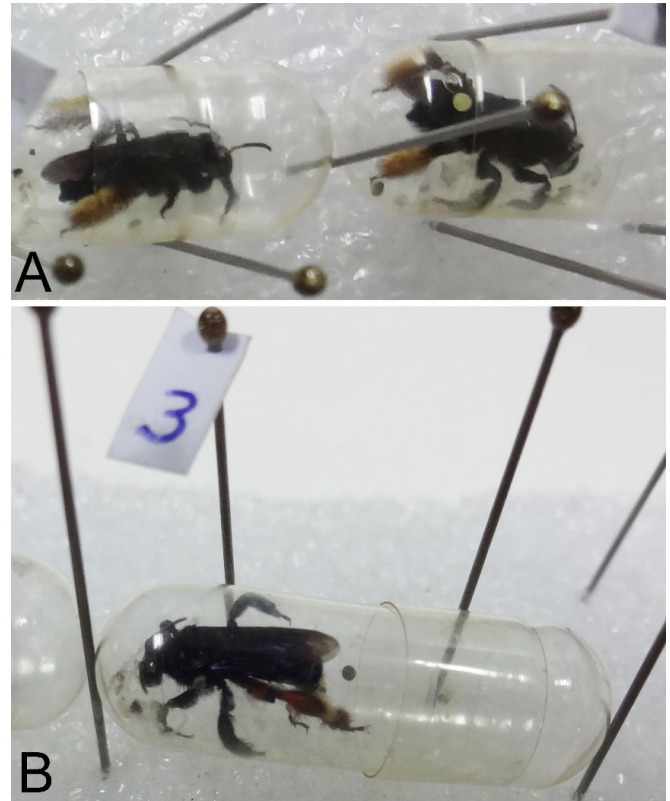
### Pollinic analysis of the residues of male and female cells

Nests built in plastic straw TNs ( $n = 24$ ) were opened 17 days after being sealed by the bee when the larvae present in the first cells had already defecated (Fig 3A). A total of 113 brood cells were opened and the contents (larvae and feces) were collected and packed individually in different containers. All nests were opened without damaging the inner walls that divide the cells (Fig 3A), then the immatures were transferred to the capsules (Fig 3B) and the feces samples from the interior of the cells were collected for analysis. To remove fecal remains, a spatula and an iron rod fixed to a toothpick were used. These were sanitized with 70% alcohol and then sterilized in flame (Fig 3C) before and after each collection, to avoid cross-contamination between samples. Before removing the feces, in each sample, a micro drop of alcohol was added so that the feces were moist but not diluted (Fig 3D), then each sample was removed with the spatula (Fig 3E) and deposited in different falcon tubes containing 2 ml of 70% alcohol (Fig 3F). Then, each tube was identified according to nest of origin and cell position in the nest. With this, we tried to verify if the female, when providing brood cells, did some differentiation about the diversity of types of pollen collected to supply the cells where males or females would develop. The collected samples were submitted to the acetolysis process (Erdtman, 1960; Silva et al., 2014). Individuals transferred to the capsules were sexed when they completed their development and became adults (Fig 4A-B). In this way pollen residues were analyzed from 97 young cells whose bees completed their development, of these 31 cells containing male larvae and 66 cells with female larvae were analyzed. Feces collected were processed and slides mounted. Quantitative and qualitative analyzes were performed as described in the previous topic.

### Data analysis

The qualitative analysis of the pollen grains presents in feces to identify their plant species was made through a comparison with the pollen grains deposited at the Palinoteca of the Laboratório de Abelhas of the Departamento de Zootecnia, UFC, specialized literature (Miranda & Andrade, 1990) and the pollen collection of the Online Pollen Catalogue Network ([www.rcpol.org.br](http://www.rcpol.org.br)).

The quantitative analysis was performed by counting the first 400 pollen grains present in each sample (Montero & Tormo, 1990), separating them by pollen type according to the concept established by Joosten and de Klerk (2002) and de Klerk and Joosten (2007). Then, the percentages of each pollen type were determined according to frequency class definitions followed the adaption by Novais et al. (2009) of the method of Louveaux et al. (1978): very constant pollen ( $> 75\%$ ); constant pollen ( $> 50\% - \leq 75\%$ ); low constancy pollen ( $> 25\% - \leq 50\%$ ); occasional pollen ( $\geq 5\% - \leq 25\%$ ) and rare pollen ( $< 5\%$ ).



**Fig 4.** Adult individuals of *Tetrapedia diversipes* Klug after completing their development inside the biodegradable capsules in the Bee Unit at the Campus do Pici/Federal University of Ceara, Fortaleza-CE, Brazil: Females (A). Male (B).

To characterize the diet of *T. diversipes*, the trophic niche breadth was calculated by the Shannon Diversity Index ( $H'$ ) (Shannon, 1948). This index ponders richness and abundance of pollen types and how much each one was used. Confidence intervals for the Shannon indices were calculated using a bootstrap procedure. The supplied number of random samples ( $n = 9999$ ) was produced, each with the same total number of individuals as in the original sample. For each individual in the random sample, the taxon was chosen with probabilities proportional to the original abundances. A confidence interval of 95% was calculated. The non-overlap of the confidence intervals implies that the indices differ from each other for a significance of 5%. The Pielou equitability Index was used to investigate if the pollen types of the different plant species were used uniformly (Pielou, 1966). This index varies from 0 to 1, corresponding to irregular visits to plant species until a uniformity of the visitation to collect food resources.

We also applied the Berger-Parker dominance index (Magurran, 2004) to verify the occurrence of the most dominant (abundant) pollen types in the food samples. We used the same analysis, described above, to characterize the trophic niche breadth of the immature diet of *T. diversipes*, according to sex. Subsequently, we investigated the diet overlap between the sexes using the Morisita Index – Horn (CH). We then compared the frequency distribution of food items between the two sexes was compared by the Kolmogorov-Smirnov test (ZAR, 1999).



The analyses were made in the statistical program PAST 2.17c (Hammer et al., 2001). Moreover, to verify differences in the composition of pollen types used by females to provide cells according to sex, we used permutational multivariate analysis of variance (PERMANOVA, function: adonis, package: vegan) and Multivariate homogeneity of groups dispersions (PERMDISP, function: betadisper, package: vegan). Both analyses were based on Bray-Curtis dissimilarity calculations, to determine whether the groupings were significantly different in location (turnover beta diversity) and dispersion (variation beta diversity) in a multivariate space (Anderson et al., 2011; Avolio et al., 2019). A difference in location (distances between centroids) would indicate that the resources used to provide cell was significantly different in composition according to sex. Because the PERMANOVA method may confound location and dispersion effects, we tested for homogeneity of dispersion (distances of samples from their centroid). Therefore, not significant differences in dispersion paired with a significant difference according to the PERMANOVA would indicate that the pollen type composition differed between groups. Also, we used data visualization (ordination plot-PCoA and boxplot derived from betadisper function) to support the interpretation of statistical tests. The pollen types driving ordination were determined using the envfit function (package: vegan, permutations: 999) and fitted to the plot according to their significant effect ( $p < 0.05$ ) on ordination. Moreover, we wished to determine for temporal changes in the pollen types composition according to sex. Therefore, the PERMANOVA was conducted with terms sex, month and sex by month interaction. We excluded data from July because only female emerged from cells. As several samples correspond to same nest we account for dependency using nest ID as strata within which to constrain permutations (999

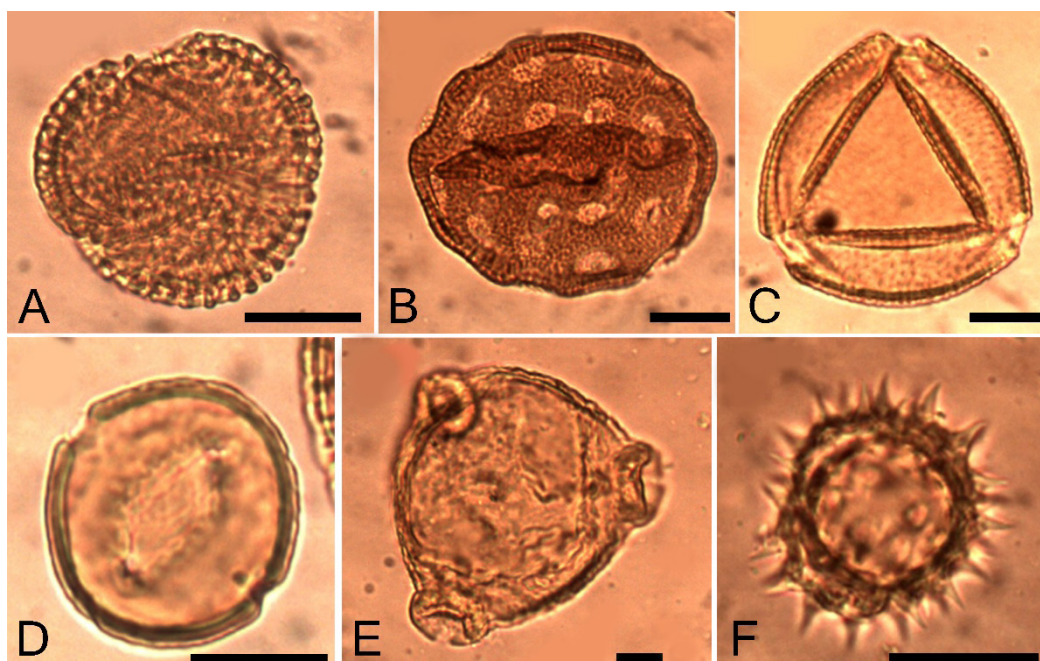
iterations). Analyses and graphs were completed in R version 4.0.3 (R Core Team, 2020).

## Results

### Trophic niche

The analysis of feces samples of TNs ( $n = 49$ ) showed that the immature diet was composed of pollen from 29 plant species belonging to 16 botanical families (Table 1). Only five pollen types were not identified, representing 2.38% of the total samples analyzed. *Dalechampia pernambucensis* (Euphorbiaceae) (Fig 5A), composing 59.57% of all pollen samples analyzed was the pollen type considered constant. Monthly, its percentage participation ranged from 15.95% to 84.15% in the immatures' diet.

The bees' diet changed throughout the year. Considering the number of species used in the immatures' diet, May was the month with the greatest number of pollen type explored ( $n = 26$ ), and the most frequent pollen types were *Talinum fruticosum* (Talinaceae) (20.70%), *D. pernambucensis* (18%), *Ludwigia* type. (Onagraceae) (Fig 5E) (13.50%), and *Tithonia diversifolia* (Asteraceae) (Fig 5F) (10.80%). The other 22 pollen type found in the May diet showed percentages below 10% (Table 1). In March only seven species composed the immatures' diet and *D. pernambucensis* (Euphorbiaceae) pollen grains were predominant (84.15%), followed by *T. fruticosum* (Fig 5B) (12.85%), *Turnera subulata* (Turneraceae) (Fig 5C) (2.65%), and the remaining 0.35% were composed of four different pollen type (Table 1). During December, the most frequent pollen grains were *Malpighia emarginata* (Malpighiaceae) (Fig 5D) (35.80%), considered a source of floral oil and sporadically a source of pollen, and *T. subulata* (33.50%), being considered as a supplier of nectar and pollen (Table 1).



**Fig 5.** Major pollen types used in the diet of immature *Tetrapedia diversipes* Klug in the Bee Unit at the Campus do Pici/ Federal University of Ceara, Fortaleza-CE, Brazil: *Dalechampia pernambucensis* (A), *Talinum fruticosum* (B), *Turnera subulata* (C), *Malpighia emarginata* (D), *Ludwigia* type (E) and *Tithonia diversifolia* (F). Scale bars 20 $\mu$ m.

**Table 1.** Percentage participation of pollen types identified in the residual samples of *Tetrapedia diversipes* Klug nests from December 2014 to September 2015, in the Bee Unit at the Campus do Pici/Federal University of Ceara, Fortaleza-CE, Brazil. Resources Available (RA), Pollen (P), Nectar (N), Oil (O). Number of species (S), Diversity Index (H'), Equitability Index (J') and Dominance Index (D). The outstanding figures are highlighted in bold numbers. \*Pollen types for which their families unidentified. \*\*Resources based on genus.

Family/ Pollen types	Monthly percentage of pollen types												Total	
	December	January	February	March	April	May	June	July	August	September	%	RA		
<b>AMARANTHACEAE</b>														
<i>Alternanthera brasiliana</i>					0.05	0.10	0.10	0.46	0.11	0.03	0.08	P/N		
<b>ASTERACEAE</b>														
<i>Asteraceae</i> type	0.20			0.10		8.25	0.04				0.73	-		
<i>Mikania cordifolia</i>							2.92	0.04	0.04		0.30	P/N		
<i>Tithonia diversifolia</i>	0.50					<b>10.80</b>	1.71	0.38	0.11	0.05	1.19	P/N		
<i>Elephantopus</i> type		0.15	0.05			1.55					0.25	P/N**		
<b>BIGNONIACEAE</b>														
<i>Tecoma stans</i>		0.05			0.10						0.01	P/N		
<b>EUPHORBIACEAE</b>														
<i>Croton</i> type 1						0.20					0.02	P/N**		
<i>Croton</i> type 2	0.10					0.25					0.03	P/N**		
<i>Dalechampia pernambucensis</i> .	<b>15.95</b>	<b>68.35</b>	<b>75.05</b>	<b>84.15</b>	<b>63.95</b>	18.00	<b>62.13</b>	28.42	<b>72.00</b>	<b>84.03</b>	59.57	P/N		
UNIDENTIFIED TYPES														
Type 1*	0.15		4.30		1.35	6.75	1.67	1.46	0.54	4.45	2.20	-		
Type 2*						0.20		0.04			0.02	-		
Type 3*					0.95	0.50					0.12	-		
Type 4*						0.15					0.01	-		
Type 6*							0.33				0.03	-		
<b>FABACEAE/CAESALPINOIDEAE</b>														
<i>Bauhinia</i> type	0.25	0.10				0.20	5.08	24.79	2.93	1.28	3.65	P/N**		
<b>FABACEAE/MIMOSOIDEAE</b>														
<i>Leucaena leucocephala</i>						0.10	0.67				0.08	P/N**		
<b>FABACEAE/PAPILIONOIDEAE</b>														
<i>Libidibia ferrea</i>						0.10					0.01	N		
<b>LORANTHACEAE</b>														
<i>Struthanthus syringifolius</i>	0.05			0.15	0.10	0.20				0.05	0.05	P/N		

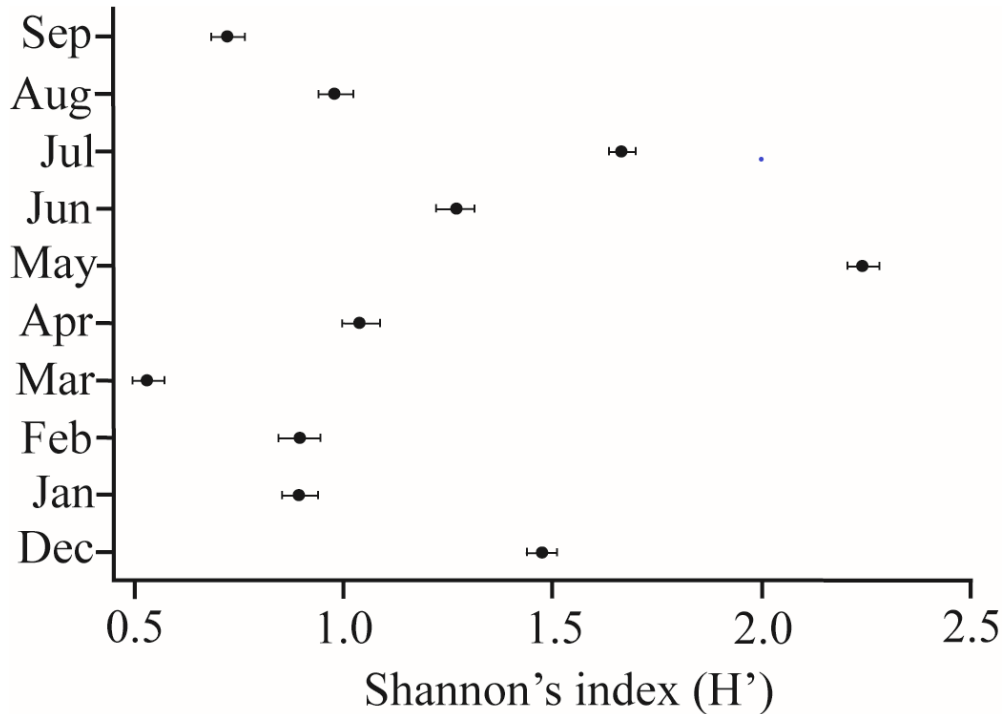
**Table 1.** Percentage participation of pollen types identified in the residual samples of *Tetrapedia diversipes* Klug nests from December 2014 to September 2015, in the Bee Unit at the Campus do Pici/Federal University of Ceara, Fortaleza-CE, Brazil. Resources Available (RA), Pollen (P), Nectar (N), Oil (O). Number of species (S), Diversity Index (H'), Equitability Index (J') and Dominance Index (D). The outstanding figures are highlighted in bold numbers. \*Pollen types for which their families unidentified. \*\*Resources based on genus. (Conclusion)

Family/ Pollen types	Monthly percentage of pollen types												Total	
	December	January	February	March	April	May	June	July	August	September	%	RA		
<b>MALPIGHIACEAE</b>														
<i>Byrsonima crassifolia</i>			1.00		0.10	0.05			0.04	3.95	0.77	P/O		
<i>Malpighia emarginata</i>	<b>35.80</b>	0.10	0.20	0.05	5.05	0.60	0.38	0.75	0.07		3.67	P/O		
<b>MALVACEAE</b>						0.05					0.00	N**		
<i>Malvaceae</i> type														
<b>MYRTACEAE</b>														
<i>Psidium guajava</i>						0.15				0.03	0.02	P		
<b>NYCTAGINACEAE</b>														
<i>Boerhavia diffusa</i>	0.30	3.25		0.05	0.10	3.10					0.58	P/N		
<b>ONAGRACEAE</b>														
<i>Ludwigia</i> type			1.30		0.05	13.50	15.88	<b>20.21</b>	7.32	3.33	6.36	P/N**		
<b>TALINACEAE</b>														
<i>Talinum fruticosum</i>	3.05	<b>24.15</b>	13.30	12.85	<b>24.90</b>	20.70	3.96	19.17	14.25	0.75	12.56	P/N		
<b>RUBIACEAE</b>														
<i>Borreria spinosa</i>						0.05			0.11	0.03	0.02	P/N		
<i>Richardia grandiflora</i>	9.75	2.65	0.30		3.20	12.05	8.54			0.10	3.25	P/N		
<b>SOLANACEAE</b>														
<i>Solanum paniculatum</i>	0.40	0.05				1.15				0.18	0.17	P		
<b>TURNERACEAE</b>														
<i>Turnera subulata</i>	<b>33.50</b>	1.15	4.50	2.65	0.10	1.25		1.04	1.64	1.78	4.26	P/N		
<i>Tixa</i> (S)	13	10	9	7	13	26	9	13	13	14				
<b>Shannon (H')</b>	1.474	0.893	0.895	0.529	1.038	2.240	1.269	1.663	0.978	0.721				
<b>Pielou (J')</b>	0.575	0.388	0.407	0.272	0.405	0.688	0.578	0.648	0.381	0.273				
<b>Berger-Parker (D)</b>	0.358	0.684	0.751	0.842	0.640	0.207	0.621	0.284	0.720	0.840				

The diversity index values ( $H'$ ), equitability index ( $J'$ ) and dominance level ( $D$ ) are shown in Table 1. The confidence intervals (CI) for the diversity indices are summarized in Fig 6. A non-overlapping of the CIs implies that the indices differ from each other for a significance of 5%. In March and September, *D. pernambucensis* represented more than 80% of the diet of *T. diversipes*; these values directly influenced the values of the Berger-Paker indexes that obtained the two highest values, respectively,  $D = 0.842$  and  $D = 0.840$ . The great dominance of *D. pernambucensis* in the immature's diet, about to the other plant species, provided lower values

for diversity index ( $H' = 0.529$  and  $H' = 0.721$ ). This happened because the female bees concentrated on foraging in few botanic species to obtain the provision for their offspring.

We observed that as the percentage participation of *D. pernambucensis* decreased in the diet, there was an increase in the number of species used in more balanced proportions, clearly evidenced in December, May and July, in which the participation of the pollen of *D. pernambucensis* was respectively 15.95%, 28.42% and 18.00%. In these months, the diversities ( $H'$ ) were the highest ( $H'_{Dec} = 1.474$ ,  $H'_{May} = 1.663$  and  $H'_{Jul} = 2.24$ ), respectively.



**Fig 6.** Point and interval estimates (by bootstrap) for Shannon's  $H'$  diversity indices of the pollen types identified in the residual samples of *Tetrapedia diversipes* Klug nests from December 2014 to September 2015, in the Bee Unit at the Campus do Pici/Federal University of Ceara, Fortaleza-CE, Brazil.

#### Pollinic resources provided in cells

We found differences between females ( $n = 66$  brood cells) and males' ( $n = 31$  brood cells) diet. The females' diet was composed of 22 pollen type, while that of the males' diet was composed of 27 (Table 2). Individually, the cells of females contained among 2 to 12 pollen types ( $\bar{X} = 6.2 \pm 2.1$ ) and males with 3 to 12 pollen types ( $\bar{X} = 6.6 \pm 2.6$ ). When we compared the trophic niche breadth between the two sexes, we noticed that males presented a diet less diverse than females ( $H'_M = 1.08$ ,  $H'_F = 1.32$ ;  $t = 16.371$ ;  $p < 0.0001$ ).

There was a high overlap in the use of plant species as source of pollen, 98.9% ( $CH = 0.989$ ) between the two sexes, result confirmed by multivariate analyses (PERMANOVA, PERMDISP, PCoA); however, there was no statistical difference

in the frequency distribution of the food items of males and females ( $D = 0.214$ ,  $p = 0.341$ ). The pollen type composition between cells according to sex had equal dispersion (PERMDISP:  $F_{1,91} = 1.42$ ,  $p = 0.24$ , Fig 7) and no significant difference in location according to sex (PERMANOVA:  $R^2 = 0.01$ ,  $F_{1,85} = 0.99$ ,  $p = 0.42$ , Fig 7), month (PERMANOVA:  $R^2 = 0.44$ ,  $F_{3,85} = 23.41$ ,  $p = 0.40$ ) and their interaction (PERMANOVA:  $R^2 = 0.02$ ,  $F_{3,85} = 1.31$ ,  $p = 0.09$ ). However, the PCoA allowed us to confirm that the females used fewer resources to provide male cells to the female cells (Fig 7). Of the pollen types identified, only seven were not used by both sexes. The pollen types of *D. pernambucensis* and *T. fruticosum* were the most frequent in both female and male diets. Another important plant species was *Ludwigia* type. (Onagraceae); however, it had a higher proportion in the female diet (Table 2).



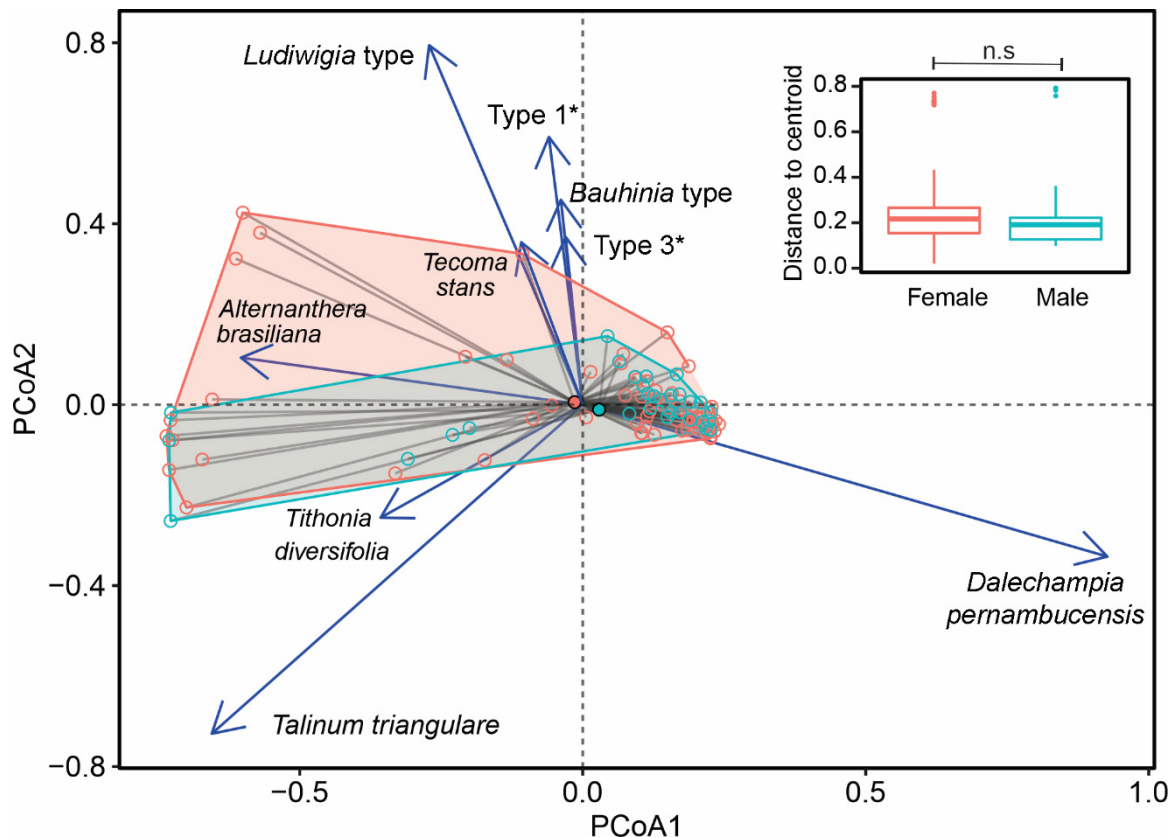
**Table 2.** Pollen types identified in the diet provided for male and female offspring of *Tetrapedia diversipes* Klug, from June to October 2015, in the Bee Unit at the Campus do Pici/Federal University of Ceara, Fortaleza-CE, Brazil. Absolute Frequency (AF) and Relative Frequency (RF). The outstanding figures are highlighted in bold numbers. \*Pollen types for which their families unidentified.

Family	Pollen types	♀		♂	
		AF	RF (%)	AF	RF (%)
Amaranthaceae	<i>Alternanthera brasiliana</i>	59	0.22	18	0.15
Asteraceae	<i>Asteraceae</i> type	27	0.10	0	0.00
	<i>Elephantopus</i> type	159	0.60	72	0.58
	<i>Emilia sonchifolia</i>	0	0.00	1	0.01
	<i>Mikania cordifolia</i>	0	0.00	2	0.02
	<i>Tithonia diversifolia</i>	288	1.09	157	1.27
Bignoniaceae	<i>Tecoma stans</i>	74	0.28	23	0.19
Fabaceae	<i>Phaseolus</i> type	0	0.00	5	0.04
Euphorbiaceae	<i>Croton</i> type	0	0.00	1	0.01
	<i>Dalechampia pernambucensis</i>	16609	<b>62.91</b>	8884	<b>71.65</b>
Unidentified Types	Type 1*	899	3.41	321	2.59
	Type 2*	16	0.06	12	0.10
	Type 3*	37	0.14	10	0.08
	Type 4*	2	0.01	1	0.01
	Type 5*	0	0.00	1	0.01
	Type 6*	11	0.04	1	0.01
Fabaceae/Caesalpinioideae	<i>Bauhinia</i> type	1214	4.60	79	0.64
Fabaceae/Mimosoideae	<i>Leucaena leucocephala</i>	12	0.05	1	0.01
Loranthaceae	<i>Struthanthus syringifolius</i>	19	0.07	7	0.06
Malpighiaceae	<i>Byrsonima crassifolia</i>	179	0.68	48	0.39
	<i>Malpighia emarginata</i>	146	0.55	8	0.06
Myrtaceae	<i>Psidium guajava</i>	2	0.01	3	0.02
Onagraceae	<i>Ludwigia</i> type	2950	<b>11.17</b>	1139	9.19
Talinaceae	<i>Talinum fruticosum</i>	3408	<b>12.91</b>	1296	<b>10.45</b>
Rubiaceae	<i>Borreria spinosa</i>	45	0.17	10	0.08
	<i>Richardia grandiflora</i>	6	0.02	1	0.01
Solanaceae	<i>Solanum paniculatum</i>	0	0.00	16	0.13
Turneraceae	<i>Turnera subulata</i>	238	0.90	283	2.28
<b>Total</b>		26400	100	12400	100

## Discussion

*Tetrapedia diversipes* presented a broad trophic niche, in which the females provisioned the cells with resources from flowers of 29 plant species: *D. pernambucensis* Baill. (Euphorbiaceae), *T. fruticosum* (L.) Juss. (Talinaceae), *Ludwigia* sp. (Onagraceae), *T. diversifolia* (Hemsl.) A. Gray (Asteraceae) and *Turnera subulata* Sm. (Turneraceae) were more explored for pollen collection, characterizing this species as presenting polylectic habit (Muller, 1996; Ferreira et al., 2019). However, the number of plant species used to feed the immatures was lower in the present study in comparison to other studies with this same bee species in other vegetation types in different regions of Brazil (Menezes et al., 2012; Neves et al., 2014). Menezes et al. (2012) analyzed the pollen sources used in the diet of

immature *T. diversipes* in two areas of the Atlantic Forest, one consisting of 215 ha of eucalyptus plantation interspersed with native vegetation and the other with 2,400 ha of dense ombrophilous forest vegetation. In these areas the authors identified 20 types of pollen (nine exclusive types) and 18 pollen types (with seven exclusive types) respectively. In both areas, the authors identified that *Dalechampia* type. was the predominant pollen type. Neves et al. (2014) identified in a tropical agroecosystem a total of 60 pollen types in the diet of the *T. diversipes*, in which the predominant pollen type was Euphorbiaceae (60.5%), followed by Malpighiaceae (16.8%) and Asteraceae (12.2%). These authors found that *Dalechampia dioscoreifolia* was a predominant pollen type in the diet of the larvae of *T. diversipes* and indicates the importance of this plant in maintaining populations of this solitary bee.



**Fig 7.** Principal Coordinates Ordination based on Bray-Curtis distances comparing the pollen type composition according to sex of cells provided by *Tetrapedia diversipes* Klug. Filled circles represent the centroid of groups and empty circle sample of each group. The boxplot (median and quartiles) shows the sample-to-Centroid distance for each sex. Arrows indicate the most significant pollen types ( $p < 0.01$ ) driving the ordination of points. The arrow length corresponds to the strength of the correlation between the pollen types and the ordination.

In the present study, *T. diversipes* concentrated their foraging in *D. pernambucensis*, since this pollen type represented more than 50% of the pollen grains sampled. The use and preference of floral resources from this plant species by *Tetrapedia* bees corroborate the results found in other studies (Menezes et al., 2012; Neves et al., 2014; Rocha-Filho & Garófalo, 2015).

In this study, *T. fruticosum* pollen was considered an important species in the diet of *T. diversipes*, this species is listed as an important food resource for bees from the Caatinga, being visited mainly by solitary bees (Maia-Silva et al., 2012), however, its use by *T. diversipes* had not yet been registered. This plant is a perennial herbaceous species well suited to hot and humid climates and low fertility soils and is considered an invasive plant species (Souza & Lorenzi, 2005).

The study area presents edaphic climatic conditions favorable to the presence of plants such as *T. fruticosum*, which are not very demanding and possibly these conditions allowed *T. diversipes* to find in this species floral resources in great availability and perenniality evidenced by the presence of the pollen types throughout the study period, with percentage variation from 0.75 to 26.90% in diet composition. However, this fact could also be interpreted as a preference of this bee species for this pollen host.

The *Byrsonima crassifolia* and *Malpighia emarginata* (Malpighiaceae) pollen types probably indicate or point to sources of oil, because the grains were recorded in low percentages on the slides analyzed. However, in December, the pollen type *M. emarginata* was considered an important source of pollen for the immature diet representing 35% of the pollen grains in the samples.

In our observations, despite the pollen of *D. pernambucensis* and *T. fruticosum* predominated in the analyzed samples, as well as the presence of *D. pernambucensis* in the diet of *T. diversipes* is recurrent in the literature, this does not classify this bee as an oligolectic species since according to Muller (1996) and Werneck et al. (2015) a bee is only classified as oligolectic when the frequency of dominant pollen in its diet corresponds to at least 95%. In our studies, the frequency of these pollen types did not reach 90%.

Genuinely oligolectic bees can present morphological and/or behavioral adaptations that allow them to use the floral resources provided by plants more efficiently when compared to the polylectic competitors (Pinheiro & Schlindwein, 1998; Alves-dos-Santos & Wittmann, 1999; Alves-dos-Santos, 2003; Alves-dos-Santos et al., 2006). If in this relationship, adaptations are demonstrated that benefit both, this bee-plant interaction may have evolved together, so that one depends on the other

to remain in the environment (Williams, 2003; Schlindwein, 2004; Schlindwein & Medeiros, 2006; Larkin et al., 2008). Considering the enormous complexity in determining/classifying a species as oligolectic and/or polylectic Silva et al. (2016) used the term “temporal preference” to characterize a high pollen frequency of *Miconia chamissois* (Melastomataceae) in the diet of *Euglossa towsendi* Cockerell, 1904 for a limited period of time and Ferreira et al. (2019) used the term “temporary specialization event” to explain the preference of *Ancylloscelis apiformes* for pollen types of Malpighiaceae and Convolvulaceae species under the same circumstances. According to the theory of optimal foraging where the nutritional value and the energy contained in the food collected exceed the energy spent during its collection, the lower number of plant species used by *T. diversipes* in this study may be due to the differences in vegetation and the land use in the different areas studied; the distance between nesting sites and food resources and the individual preferences of each foraging female (Levin, 1978; Zimmerman, 1988).

The individual analysis of the immature feces contained within the *T. diversipes* brood cells showed that they visit more plant species to supply cells where males have developed than in cells where females have developed, this difference indicates that the bee probably has specific receptors that regulates the nutrients offered among the various food sources explored (Ruedenauer et al., 2020). Although, the pollen sources used were similar between the sexes, the richness of pollen types found in the diet of males and females indicates that the founding bees used similar plants in the composition of the pollen mass, but since the differences between the sexes were low, probably mixing these types in the same cell possibly indicates a balance similar for the development of immatures of both sexes. However, the assessment of the nutritional value of this diet probably reveals some difference in the composition and amount of nutrients offered to males and females. Our results indicate that *T. diversipes* concentrated the foraging on specimens of *D. pernambucensis* to provision their cells, since this pollen type composed most of the diet both for male and female offspring.

The Onagraceae family was considered important in the residual samples of both sexes, being represented by the pollen type *Ludwigia* type. This pollen type has already been pointed out as an important source for the diet of *T. diversipes* in studies made from the analysis of residual pollen in nests by Menezes et al. (2012).

Finally, we can conclude that this study brings a contextualized approach to the trophic niche from the post-emergence residue of solitary bees *Tetrapedia diversipes* in a tropical environment and Semi-deciduous Lowland Forest vegetation. Although *T. diversipes* presents an oligolectic tendency in the collection of resources, it was verified that the bee expands its trophic niche, and sometimes proves to be polylectic in pollen collection, taking advantage of the resources available in the vicinity, in a strategy defined as

temporal preference. Further studies on the diet of this species are necessary, aiming at the maintenance of this bee in the environment, through a better knowledge of the species of plants it uses.

### Authors' Contributions

Conceptualization AMC, BMF and CIS. Material preparation, and data collection were performed by AMC, GSP, DSN and MCP. The identification of pollen types was performed by CIS. The data analysis and first draft of the manuscript was performed by AMC and all authors commented on previous versions of the manuscript. Supervision was provided by BMF and CIS. All authors read and approved the final manuscript.

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### Disclosure statement

The authors declare that they have no potential conflict of interest in relation to the study in this paper.

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