Nesting and use of pollen resources by *Tetrapedia diversipes* Klug (Apidae) in Atlantic Forest areas (Rio de Janeiro, Brazil) in different stages of regeneration

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ABSTRACT. Nesting and use of pollen resources by *Tetrapedia diversipes* Klug (Apidae) in Atlantic Forest areas (Rio de Janeiro, Brazil) in different stages of regeneration. The nesting in trap-nests and use of pollen sources in larval food by *Tetrapedia diversipes* Klug, 1810 (Apidae) was compared between regenerating areas of Atlantic Forest. The study was conducted between April 2008 and October 2009 at União Biological Reserve, Rio de Janeiro, Brazil. *T. diversipes* nested in 66 trap-nests and showed a peak of nesting during the months of highest rainfall. The most frequent pollen type in brood cells during the wet season was *Dalechampia* sp. 1. During the dry season, the type *Ludwigia* sp. was the most frequent, followed by *Dalechampia* sp. 2. The high frequency of *Dalechampia* and *Ludwigia* species in the larval food, observed in both habitats and in the two seasons could be considered relevant for *T. diversipes*, suggesting highly selective diet based primarily on two plant species unrelated, but similar in size of pollen grains.

KEYWORDS. Atlantic rainforest; Oil collecting bee; Pollen analysis; Tetrapediini; Trap-nests.

RESUMO. Nidificação e uso de fontes de pólen por *Tetrapedia diversipes* Klug (Apidae) em áreas de Mata Atlântica (Rio de Janeiro, Brasil) em diferentes estágios de regeneração**.** A nidificação em ninhos-armadilha e o uso de fontes de pólen no alimento larval por *Tetrapedia diversipes* Klug, 1810 (Apidae) foram comparados entre áreas de Floresta Atlântica em regeneração. O estudo foi realizado entre abril de 2008 e outubro de 2009 na Reserva Biológica União, Rio de Janeiro, Brasil. *T. diversipes* nidificou em 66 ninhos-armadilha e apresentou um pico de nidificação durante os meses de maior precipitação pluviométrica. O tipo polínico mais frequente nas células de cria durante a estação chuvosa foi *Dalechampia* sp. 1. Durante a estação seca, o tipo *Ludwigia* sp. foi o mais frequente, seguido de *Dalechampia* sp. 2. A alta frequência de espécies de *Dalechampia* e *Ludwigia* no alimento larval, observada em ambos os habitats e nas duas estações deve ser considerada um fato relevante para *T. diversipes*, sugerindo uma dieta altamente seletiva baseada primariamente em duas espécies vegetais não relacionadas, porém semelhantes quanto ao tamanho dos grãos de pólen.

PALAVRAS-CHAVE. Abelha coletora de óleo; Análise polínica; Floresta Atlântica; Ninhos-armadilha; Tetrapediini.

Oil-collecting bees in the Neotropics belong to three tribes: Centridini, Tapinotaspidini and Tetrapediini (Alves-dos-Santos *et al.* 2007). Traditionally Tetrapediini bees include two genera: *Tetrapedia*, composed by oil-collecting and nest-constructing bees and *Coelioxoides*, cleptoparasite species of *Tetrapedia* (Alves-dos-Santos *et al.* 2002) although recent phylogenetic analyses do not confirm the monophyly of the tribe (Cardinal *et al.* 2010).

The genus *Tetrapedia* comprises solitary bees widely distributed in tropical regions of the Americas and represented by 18 species in Brazil (Moure 2008). Their females commonly nest in cavities in wood, and they may also occupy the inactive nests of other Hymenoptera such as *Anthodioctes megachiloides* Holmberg, 1903 (Megachilidae) and *Trypoxylon* (Crabronidae) (Alves-dos-Santos *et al.* 2002). The reuse of cells from abandoned nests of *Polistes simillimus* Zikán, 1951 (Vespidae) by *Tetrapedia diversipes* Klug, 1810 has also been observed (Pinto 2005). In addition to building its nests in pre-existent wood cavities, *T. diversipes* can also be attracted to trap-nests (Roubik 1987; Alves-dos-Santos *et al.* 2002; Garófalo *et al.* 2004; Camillo 2005). This sampling methodology gives access to significant information about nesting species, such as nesting biology, building materials, architecture, and food resources provided for larvae (Garófalo *et al.* 2004).

Although the biology of *T. diversipes* is well known (Alves-dos-Santos *et al.* 2002; Camillo 2005), reports of interaction between this species and food sources are poorly described in the literature, as well as information about its importance as a pollinator. Analysis of the pollen in larval food gives precise information about the range of plants visited and the relative importance of those sources to the bees (Dórea *et al.* 2010).

Bees are important components of biological communities, not only because of their role as pollinators, but also because they can be very sensitive to the effects of environmental disturbances (Morato & Campos 2000; Steffan-Dewenter 2003; Holzschuh *et al.* 2010). Among bees, species that specialize in the collection of floral oils compose an important guild of pollinators associated with specific plant groups in several ecosystems, such as the Atlantic Forest, cerrado and restinga (Teixeira & Machado 2000; Gaglianone 2003, 2006; Dunley *et al.* 2009). Currently, only approximately 7% of the original extent of the Atlantic Forest remains. This is one of the biomes most affected by deforestation (Myers *et al.* 2000; Morellato & Haddad 2000). In the state of Rio de Janeiro, the process of forest devastation and fragmentation has been quite intense, and currently 18,4% of its original vegetation cover is in either privately or publicly held conservation areas (SOS Mata Atlântica/INPE 2010).

Due to the reduction of natural areas in recent decades, the study of biological processes in altered ecosystems is being emphasized (Saunders *et al.* 1991; Naeem 2002). Of particular interest are insects with important ecological functions, such as predators, parasites and pollinators; these are frequently used in studies evaluating the effects of habitat loss on biodiversity (Tscharntke 1992; Kevan 1999; Steffan-Dewenter & Tscharntke 2002). However, such studies involving the Atlantic Forest are still rare (Viana *et al.* 2006; Loyola & Martins 2008, 2009). Comparing nesting parameters in Atlantic Forest areas in different stages of regeneration can indicate preferences and fidelity to certain habitats, providing important information about the handling and conservation of these bees.

The objective of this study is to compare the use of Atlantic Forest habitats in different stages of regeneration by *T. diversipes* bees by considering trap-nest occupation, seasonality, and sources of pollen.

MATERIAL AND METHODS

This study was carried out at União Biological Reserve (Rebio União) (22º25'40"S, 42º02'06"W), located in the municipalities of Rio das Ostras, Casimiro de Abreu and Macaé, in the northern portion of Rio de Janeiro state, Brazil, between March 2008 and October 2009. The area contains 3126 ha: 2400 ha of dense Atlantic ombrophilous forest vegetation, 215 ha of abandoned eucalyptus plantation interspersed with native vegetation, and the remaining consists of areas altered by human action (IBAMA 2007). In these former plantations, the native species are regenerating themselves and forming an understory (Evaristo 2008). The predominating vegetation is considered one of the best preserved in the coastal lowlands in Rio de Janeiro state, even though there are areas in regeneration that were altered due to selective logging, hunting, introduction of exotic species in the area (eucalyptus and fruit trees), and the construction of high tension power lines and underground ducts for the transportation of combustible materials (IBAMA 2007). The predominant climate in the region is humid tropical, with a median annual temperature of 24ºC and 1658 mm/year of rainfall, of which 75% occurs between October and April (IBAMA 2007).

Twelve sampling sites were selected at Rebio União: six in understory areas of abandoned eucalyptus plantations where there are native species in initial stage of regeneration (henceforth termed eucalyptus with regenerating understory, ERU); and six areas of secondary Atlantic Forest (SAF) in advanced stage of regeneration. An identical number of trap-nests (n = 1440) was installed at the sites monthly. These trap-nests were either bamboo canes of various diameters grouped in sets of three and attached to poles at approximately 1.5m above the ground, or black cardboard tubes varying from 4 to 15 mm in diameter and inserted in wood blocks at the same height. The trap-nests were checked monthly and replaced with new ones after they were closed by the bees. The occupied nests were brought to the laboratory, kept at room temperature and checked daily. The plant species in bloom, within a radius of 200 meters close to the nesting sites in areas of eucalyptus with regenerating understory, were also collected monthly for the development of a reference collection of pollen slides of this area.

After the *T. diversipes* adults emerged, the trap-nests were opened and the pollen content in the brood cells was removed and stored with glacial acetic acid to acetolysis (see Erdtman 1960). The cell with the highest pollen content from each nest studied was visually selected, and all the pollen content was fixed as the representative sample of the nest. After the samples were acetolyzed, three slides were prepared from each one, and the types of pollen were identified and quantified. The exclusive pollen types were considered as those found uniquely in samples obtained in one habitat and season studied. The qualitative analysis consisted of determining the genera and types of pollen (group of grains with very similar morphology) according to specialized literature (Salgado-Labouriau 1973; Roubik & Moreno 1991) and reference slides from the study area. Quantitative analysis was performed by counting 400 pollen grains per slide and 1200 grains per sampled nest.

The trap-nests occupancy rate was calculated as the ratio between the number of occupied cavities in relation to total cavities available monthly. In order to examine the relation of nesting frequency and emergence of adults per month with climate variables (rain and average temperature), the Spearman (r_s) correlation was calculated. The nesting frequency of *T. diversipes* was compared between areas using the Mann-Whitney (U) test. The sex ratio was calculated using the ratio between the number of females and the number of males that emerged from the nests.

The head width of emerging *T. diversipes* was taken measuring the distance between the external edges of the eyes at the level of the base of the antenna. The analysis of the Spearman (r_s) correlation was used to test the relation between the median width of the head and the diameter class of the cavity from which the individual emerged. The median number of cells per nest was compared between areas using the Mann-Whitney (U) test. The confidence interval for all statistical tests was 5%. The diversity of food sources visited and evenness in their use was calculated using Shannon-Wiener diversity index and evenness test, respectively, according to Magurran (2003). The similarity percentage was used to calculate the similarity of food sources visited between areas, according to Krebs (1989).

RESULTS

Nesting and seasonality. A total of 1440 trap-nests (bamboo canes and cardboard tubes) were available monthly in the twelve sampling sites between March 2008 and October 2009. The study registered 1200 cavities occupied by solitary Hymenoptera (bees and wasps), and of those, 130 were used by oil-collecting bees. The maximum occupation rate per month (occupied trap-nests/available trap-nests) by oil collecting bees was 1.8%. *Tetrapedia diversipes* was the oil-collecting bee that nested in most of the cavities, building 66 nests, 44 of them between September and January in ERU, and the other 22 between October and June in SAF (Fig. 1). Nesting peak in both habitats was during the rainiest months (Fig. 1). There was a positive correlation between the nesting frequency of *T. diversipes* and precipitation ($r_s = 0.57$; $p < 0.05$) and temperature ($r_s = 0.66$; $p < 0.05$) in the areas of ERU. However, there was no significant correlation between these variables in SAF areas ($r_s = 0.36$; $p > 0.05$ and $r_s = 0.17$; $p > 0.05$, respectively). The total number of emerging *T. diversipes* individuals was 64 males and 53 females in ERU areas (sex ratio 1:0.83), and 33 males and 16 females in SAF areas (sex ratio 1:0.48). Most of *T. diversipes* adults emerged between November 2008 and April 2009 in both habitats, and the least productive months were August, September and November 2009 (Fig. 2). There was a significant correlation between frequency of emergence and precipitation in ERU areas ($r_s = 0.45$; $p < 0.05$); however, the same result was not observed in SAF areas ($r_s = 0.40$; $p > 0.05$). No significant difference was observed in the number of individuals emerging per nest in the habitats studied (U = 140.5 $p > 0.05$) (Table I).

Nests of *T. diversipes* were built only in cardboard tubes, and the diameters used in both habitats varied from 4 to 10 mm, with no significant difference in the diameters used between habitats (U = 433.5 $p > 0.05$) (Table I). Cavities between 6 and 8 mm were the most used and included 65% of the nests built. A total of 117 individuals emerged from nests constructed in the ERU areas, and 49 from those in the SAF areas. The median head width of the emerging *T. diversipes* individuals was 2.6 ± 0.7mm. There was a significant positive correlation between the cavity size and head width of the emerging individuals ($r_s = 0.86$; $p < 0.05$). The number of cells built in nests from ERU (n = 164) was higher than that of SAF (n = 76), varying from 1 to 8 cells per nest. There was no significant difference in the median number of cells built per nest between areas (U = 443.0; $p > 0.05$) (Table I). The cells were always arrayed in rows inside the cavities, and a mixture of oil and sand was used to build the internal nest structure.

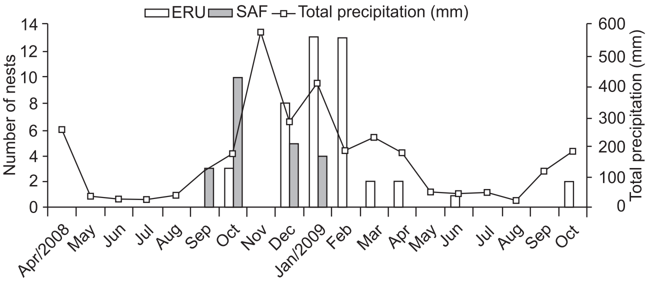

Fig. 1. Number of nests constructed by *Tetrapedia diversipes* in trap-nests, and climate data (total precipitation) between April/2008 and October/2009 in eucalyptus with regenerating understory (ERU) and secondary Atlantic Forest (SAF) areas at União Biological Reserve, Brazil.

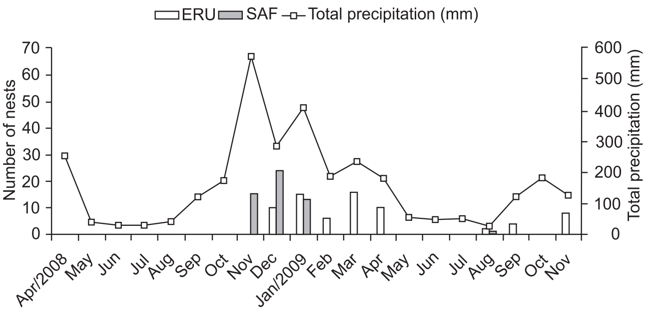

Fig. 2. Frequency of emergents of *Tetrapedia diversipes* in trap-nests, and climate data (total precipitation) between April/2008 and October/2009 in eucalyptus with regenerating understory (ERU) and secondary Atlantic Forest (SAF) areas at União Biological Reserve, RJ, Brazil.

Table I. Parameters of nests and emergents of *Tetrapedia diversipes* in the studied areas at União Biological Reserve, RJ, Brazil in eucalyptus with regenerating understory (ERU) and secondary Atlantic Forest (SAF) areas.

	ERU	SAF	p
Average diameter of the trap-nests	0.58 ± 0.01	0.60 ± 0.02	>0.05
Average number of brood cells/nest	3.7 ± 1.7 (164)	3.4 ± 1.8 (76)	>0.05
Average number of emerging/nest	3.6 ± 1.8 (117)	3.1 ± 1.5 (49)	>0.05
Sex ratio	64♂/53♀ 1:0.83	33♂/16♀ 1:0.48	>0.05/<0.0

Food resources. From nests obtained in the wet season (October 2008, December 2008, and January 2009), eighteen *T. diversipes* nests from each habitat had enough pollen to be analyzed; while in the dry season (April 2009 and June 2009) only three nests from the eucalyptus with regenerating understory area could be examined, totalling 117 pollen grain slides analyzed.

From wet season nests in ERU, 20 types of pollen (with nine exclusive types) belonging to 12 botanical families were identified (Table II); in SAF, 18 types were identified (with seven exclusive types) belonging to 11 families (Table III). Eleven types were observed in dry season nests from ERU (seven botanical families), including five exclusive types (Table IV).

The most frequent type of pollen in the wet season for both physiognomies was *Dalechampia* sp. 1 (Fig. 3A and B), with a median frequency of occurrence of 81.4% (ERU) and 90.3% (SAF) in the samples. In addition to its high frequency, *Dalechampia* sp. 1 was present in all wet season samples from both habitats considered in this study (Table II and III). The similarity percentage for resource use in both habitats during the period considered was 93.54%, even though exclusive types of pollen were observed in both habitats.

Ludwigia sp. (Fig. 3D) was the most frequent type of pollen in ERU during the dry season, followed by another species of *Dalechampia* that also appeared in this period, *Dalechampia* sp. 2 (Table IV and Fig. 3C).

According to the pollen analysis, the diversity of food resources was significantly higher in ERU than in the SAF area during the wet season (t = 42.3; df = 19; $p < 0.05$), and the use of resources was more uniform (Table V). The similarity in food resource use between the dry and wet seasons in ERU was 17.66%. During the dry season, the diversity of food resources used in the ERU area was significantly higher than that observed during the wet season in both the eucalyptus and secondary forest areas (t = 20.5; df = 19; $p < 0.05$ and t = 47.1; df = 19; $p < 0.05$, respectively).

The plant species found in bloom near the nesting sites in the ERU area were mainly herbaceous plants. There was a total of 49 species from the families: Annonaceae (1sp.), Apocynaceae (1sp.), Asclepiadaceae (1sp.), Asteraceae (9sp.), Bignoniaceae (3sp.), Capparaceae (2sp.), Celastraceae (1sp.), Convolvulaceae (2sp.), Lamiaceae (1sp.), Leguminosae Caesalpinioidea (2sp.), Leguminosae Faboidea (10sp.), Malpighiaceae (2sp.), Malvaceae (3sp.), Melastomataceae (3sp.), Myrtaceae (2sp.), Rubiaceae (2sp.), Rutaceae (2sp.) and Verbenaceae (2sp.). None of these pollen types were found in larval food from the analyzed cells.

DISCUSSION

The data obtained in the study showed *Tetrapedia diversipes* as the most frequent oil collecting bees sampled in trap-nests. This pattern was described by Garófalo *et al.*

Table II. Relative frequency per sample (I to XVIII) and average relative frequency (ARF) of pollen types in the nests of *Tetrapedia diversipes* during the wet season in eucalyptus with regenerating understory (ERU) at União Biological Reserve, RJ, Brazil. Highlighted in bold for the most frequent type in the samples.

Pollen Types	October/2008			December/2008							January/2008								ARF
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	
Anacardiaceae																			
Undetermined 1																3.6	0.1	1.4	1.7
Type *Tapirira*																0.1			0.1
Type *Schinus*													14.1	2.5					8.3
Apocynaceae																			
Type *Mandevilla*								1.0											1.0
Asteraceae					0.2								0.3			0.1		1.0	0.4
Type *Baccharis*																			
Euphorbiaceae																			
Croton sp.			0.1	0.1				0.1	0.04			0.1	0.1			0.2		0.1	0.1
Dalechampia sp. 1	**92.5**	**96.7**	**97.2**	**78.6**	**87.2**	**64.7**	**94.0**	**70.3**	**55.1**	**96.0**	**47.9**	**97.8**	**82.2**	**94.9**	**68.3**	**58.4**	**96.4**	**87.9**	**81.4**
Dalechampia sp. 2														0.1	1.2	0.5	1.7	3.6	1.4
Euphorbia sp.								8.7								0.1			4.4
Fab. (Mimosoideae)																			
Acacia sp.									0.1			0.1	0.3	0.1		0.1	0.1	0.5	0.2
Malpighiaceae																			
Type *Byrsonima*	0.2		1.4	0.4	0.7	8.7	0.4	0.8	0.1	3.6	3.2	0.9			1.6	3.0		1.8	1.9
Type *Heteropterys*	0.3	1.3	0.6	0.4	0.2	0.3		0.2	0.8	0.1	0.3	0.8	0.8	0.2	0.1		1.4		0.5
Type *Thryallis*				0.2															0.2
Melastomataceae																			
Undetermined 1																	0.1		0.1
Myrtaceae																			
Eugenia sp.			0.1															0.1	0.1
Type *Myrcia*				10.3	0.3			0.8	29.5	0.3			0.3	0.2	9.4				6.4
Onagraceae																			
Ludwigia sp.	7.0	2.0	0.7	10.2	11.3	26.3	5.7	18.1	14.3	0.1	8.4	0.1	1.9	2.0	19.3	33.9	0.2	3.1	9.2
Poaceae								0.1											0.1
Undetermined 1																			
Sapindaceae																			
Serjania sp.																		0.5	0.5
Vochysiaceae																			
Type *Vochysia*												0.2							0.2

Table III. Relative frequency per sample (I to XVIII) and average relative frequency (ARF) of pollen types in the nests of *Tetrapedia diversipes* during the wet season in secondary Atlantic Forest (SAF) at União Biological Reserve, RJ, Brazil. Highlighted in bold for the most frequent type in the samples.

Pollen types	October/2008										December/2008					January/2009			ARF
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	
Asteraceae																			
Type *Baccharis*	0.1								0.1					0.1			0.1		0.1
Type *Mikania*				1.9															1.9
Type *Taraxacum*										0.1									0.1
Euphorbiaceae																			
Croton sp.			0.1	0.1									0.1			0.1			0.1
Dalechampia sp. 1	**99.6**	**98.7**	**96.7**	**88.2**	**98.6**	**99.4**	**99.1**	**99.7**	**97.9**	**98.0**	**89.2**	**94.0**	**88.2**	**85.9**	**93.9**	**35.0**	**66.9**	**97.3**	**90.3**
Fab. (Caesalpinioideae)																			
Type *Hymenaea*														0.1					0.1
Fab. (Mimosoideae)																			
Acacia sp.		0.1		0.1				0.1	0.7						0.1		0.2		0.2
Loranthaceae																			
Struthantus sp.				0.3	0.1	0.1	0.1		0.9	0.1		0.2		0.5	0.2				0.3
Lythraceae																			
Cuphea sp.							0.1												0.1
Malpighiaceae																			
Banisteriopsis sp.					0.6	0.1	0.3	0.2	0.3	0.1	0.9	1.9	2.9	0.6	0.9	0.2	0.2	2.8	0.9
Type *Byrsonima*			0.1	0.1							0.1	0.1		7.1	1.1				1.4
Type *Heteropterys*	0.3	1.0	0.5	0.2	0.3					0.1									0.4
Type *Thryallis*															0.2				0.2
Melastomataceae																			
Type *Cambessedesia*																	0.1		0.1
Myrtaceae																			
Eugenia sp.		0.2							0.1										0.1
Type *Myrcia*				9.1	0.4	0.4	0.1		0.1			0.1		0.1		0.1	0.1		1.2
Onagraceae																			
Ludwigia sp.		0.1	2.7				0.3			1.6	9.9	3.6	8.9	5.7	3.6	32.8	0.1		6.3
Poaceae																			
Undetermined 1			0.1	0.1															0.1

Table IV. Relative frequency per sample (I to III) and average relative frequency (ARF) of pollen types in the nests of *Tetrapedia diversipes* during the dry season in eucalyptus with regenerating understory (ERU) at União Biological Reserve, RJ, Brazil. Highlighted in bold for the most frequent type in the samples.

Pollen types	April/2009		June/2009	ARF
	I	II	III	
Asteraceae				
Type *Hypochaeris*		17.1		17.1
Undetermined 1			14.1	14.1
Euphorbiaceae				
Croton sp.	0.1			–
Dalechampia sp. 2	24.8	22.4		23.6
Fab. (Mimosoidea)				
Type *Mimosa*		14.7		14.7
Malpighiaceae				
Type *Banisteriopsis*	0.2			0.2
Type *Byrsonima*	1.2	1.9		1.5
Type *Heteropterys*	0.1			0.1
Onagraceae				
Ludwigia sp.	**73.7**	**43.7**	**84.9**	**67.4**
Rubiaceae				
Type *Coffea*			1.0	1.0
Sapindaceae				
Type *Serjania*		0.1		0.1

Table V. Diversity (H') and evenness (J) of the floral resources used by *Tetrapedia diversipes* for larval supply in trap-nests in secondary Atlantic Forest (SAF) and eucalyptus with regenerating understory areas (ERU) at União Biological Reserve, RJ. Different letters indicate statistical difference (T-test at 5% significance level).

	Wet season		Dry season
	ERU	SAF	ERU
H'	0.85[a]	0.42[b]	1.19[c]
J	0.28	0.15	0.50

(2004) as occurring in other forest fragments studied in the southeast of Brazil. This relatively high abundance of nests is probably due to the low average number of brood cells observed inside the nests of this species. Alves-dos-Santos *et al.* (2002) also observed that most nests of *T. diversipes* analyzed in that study had consisted of few cells (2 to 3 cells), and, after building a nest and closing the cells, the same female looked for cavities close to the one used previously to build another nest.

The number of constructed nests was considerably higher in the eucalyptus with regenerating understory (ERU) areas than in the secondary Atlantic Forest (SAF) areas. In general, the abundance of nests built in pre-existent cavities by solitary

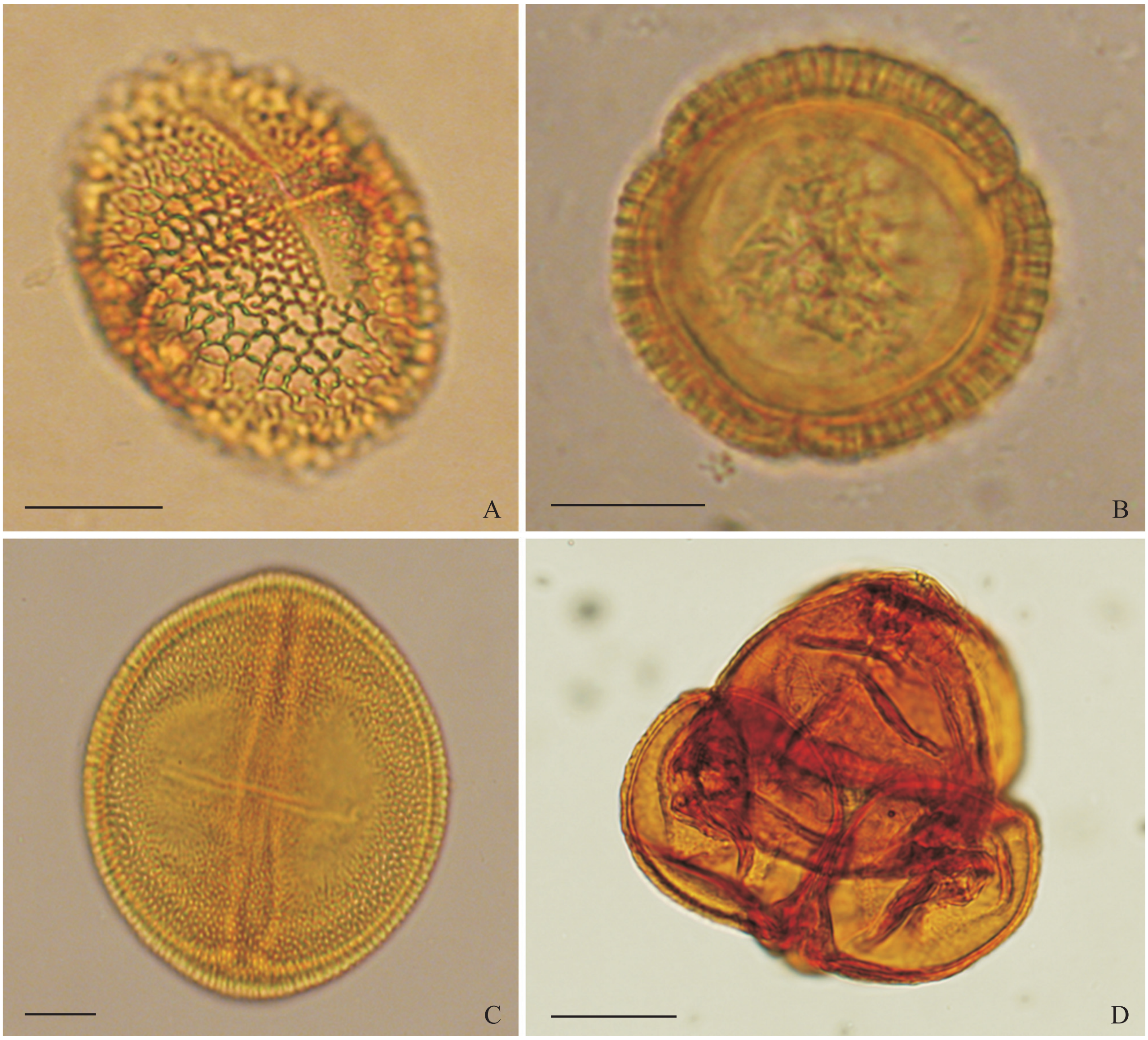

Fig. 3. Main pollen types from the larval food in nests of *Tetrapedia diversipes*. A. *Dalechampia* sp. 1 (Euphorbiaceae) in equatorial view. B. *Dalechampia* sp. 1 (Euphorbiaceae) in polar view. C. *Dalechampia* sp. 2 (Euphorbiaceae) in equatorial view. D. *Ludwigia* sp. (Onagraceae) in polar view. Scale = 20 µm.

Hymenoptera species can be related to the existence of appropriate materials for the construction and availability of food resources for adults and larvae (Roubik 1989). In addition, the availability of natural substrates in the environment for nesting has also been indicated as an influential factor in the abundance of nests in traps (Viana *et al.* 2001). We could not precise if these factors are influencing the highest number of nests in eucalyptus areas. The analysis of cell pollen content showed that most of the plants observed in bloom in this area during the *T. diversipes* nesting period were not used by these bees to feed the larvae. However, the high abundance of *Byrsonima sericea* DC (Malpighiaceae) in the eucalyptus area (Evaristo 2008) may have been attractive to this species since floral oils are necessary to build the nest cells (Alves-dos-Santos *et al.* 2002). Nevertheless, there was a low frequency of pollen grains from this plant in larval food samples, and a low frequency of *T. diversipes* females has been observed foraging in *B. sericea* flowers (Menezes & Gaglianone unpubl. data). This data suggest, therefore, that this bee species is using the eucalyptus area mainly for nesting and not for foraging.

Regarding the availability of natural substrates, the habitats considered in this study are very different in their complexity; the richness of plant species found in the secondary forest (Prieto 2008) is superior to that of eucalyptus with regenerating understory (Evaristo 2008). Even though the availability of natural cavities was not directly evaluated in this study, the higher floral richness and higher structural complexity of the forest suggest that the possibilities of natural substrate for nesting are better in the secondary forest environment. If this is indeed the case, the high frequency of nests built by *T. diversipes* in the traps installed in the area of eucalyptus with regenerating understory may be due to the greater limitation of such resources in this environment. However, *T. diversipes* is a species easily found in open habitats containing less dense vegetation, which could also justify its greater preference for the area of eucalyptus with regenerating understory. If so, the higher frequency of nests in the eucalyptus area would be expected due to the species' intrinsic preference. Studies about this species in other environments could help clarify this question.

Tetrapedia diversipes nesting activities and emergence were seasonal. There was a significant positive correlation between the frequency of these events and the environmental variables precipitation and temperature in the area of eucalyptus with regenerating understory. Even though this result did not occur in the secondary forest area, the period with the highest nesting and emergence of *T. diversipes* also corresponded to the months of the hot wet season. These data agree with findings in other regions about *T. diversipes* seasonality in forest trap-nests (Alves-dos-Santos *et al.* 2002; Alves-dos-Santos 2003). The authors reported that, in urban forest areas in São Paulo, adult females were observed to nest in the wet season, with peaks of activity from November to December and March to April (Alves-dos-Santos 2003). During the cold dry season (between June and August) bees from this species were in diapause at a mature larval stage (Alves-dos-Santos *et al.* 2002). Therefore, it was observed that despite climatic differences with the area of the present study, including the less pronounced seasonality and higher median temperatures of the latter, *T. diversipes* presented a seasonal pattern similar to that observed in areas further south in Atlantic Forest in the state of São Paulo.

It was observed strong preference of *T. diversipes* by cardboard tubes compared to bamboo canes, although the former is a non-natural substrate. A slight preference for cardboard tubes was also verified by Aguiar *et al.* (2005) and Nascimento & Garófalo (2010), but these authors found also nests of this species in bamboo canes. Thus, the type of material used for trap-nests can be suggested as an influential factor in the choice of nesting cavity. The variation in cavity diameter used by *T. diversipes* was positively correlated with the body size of these bees, justifying the high utilization of trap-nest cavities with diameters between 6 and 8 mm, which are compatible with the median body size of the individuals at Rebio União. Larger orifices would demand a greater expenditure of energy both to stock the cells and fill in gaps and, accordingly, were rarely used. A higher use of trap-nests with diameters between 6 and 8 mm was also verified by Cordeiro (2009) in areas of dense ombrophile forest.

The architecture of the nests was similar to nests from urban forest areas (Alves-dos-Santos 2003), as well as nests in semideciduous cerrado forest (*sensu lato*) (Camillo 2005), particularly regarding the size, arrangement and material used for the cells. This indicates that, regardless of the environment, these characteristics of nesting biology are preserved.

The richness of pollen sources in *T. diversipes* cells was high, as also observed by Coelho *et al.* (2010). However, the high abundance of *Ludwigia* (Onagraceae) and *Dalechampia* (Euphorbiaceae) species in the larval food observed in both habitats and in both seasons at Rebio União should be considered a relevant fact about *T. diversipes*, suggesting highly selective diet based primarily on two plant species unrelated, but similar in size of pollen grains. This does not characterized an oligolectic bee, but *Tetrapedia diversipes* surely is not a true generalist bee. The preferred plant species were not observed in the eucalyptus area, expected fact for *Ludwigia*, since their species grow preferably in flooded habitats (Ramamoorthy & Zardini 1987). Such a habitat, however, was verified at Rebio União approximately 100 to 200 m from the study area. Although *Ludwigia* has not been observed in bloom in this flooded habitat, its occurrence there is probably right. Thereby, the high abundance of this pollen type in the larval food corroborates the hypothesis that the bees are using other areas for foraging.

Silveira *et al.* (1993) and Alves-dos-Santos (1999) also reported on the interaction between *T. diversipes* and *Ludwigia* flowers in Atlantic Forest areas in the states of Minas Gerais and Rio Grande do Sul in Brazil, respectively. A study carried out by Sazima & Santos (1982) about the floral biology of the *Ludwigia sericea* (Cambess.) H. Hara in Campinas, SP, demonstrated that one species of *Tetrapedia* was the effective pollinator of this plant in the area. This was determined from pollen collecting behavior as well as the frequency and time of permanence on the flowers. Similarly, Gimenes (2002) verified the efficiency of *Tetrapedia* sp. as a pollinator of *Ludwigia elegans* (Cambess.) H. Hara in marshy areas of the Parque do Carmo in the city of São Paulo. Other reports in the literature refer to the interaction between *Tetrapedia* and Euphorbiaceae species (Armbruster & Herzig 1984; Alves dos Santos *et al.* 2002). In the former study, the authors observed visits of *Tetrapedia* sp. to *Dalechampia tiliifolia* Lam. in Panamá in which flowers these bees only collected pollen. The authors did not consider *Tetrapedia* sp. to be an effective pollinator of *Dalechampia tiliifolia*, probably because of the contrasting sizes of flowers and bees. The large size of the flowers requires large resin-collecting bees for acting as pollinators. Alves-dos-Santos *et al.* (2002) reported a high dominance of one *Dalechampia* species as well as a *Croton* species (both belong to Euphorbiaceae) observed in pollen samples from *T. diversipes* nests collected at the University of São Paulo *campus*. Although flowers of *Dalechampia* are primary sources of resin for other bees (Armbruster & Webster 1981; Armbruster & Herzig 1984), they seem to be important sources of pollen for *T. diversipes*. The high frequency of *Dalechampia*'s pollen grains in the analyzed samples and the absence of information on resin collection by *Tetrapedia* bees, reinforce the evidence that *Dalechampia* species are important sources of pollen for this bee. Other studies have described the collection of resources by *T. diversipes* from flowers of other families, such as nectar in Orchidaceae (*Oncidium paranaensis* Kraenzl.), pollen in Cactaceae (*Opuntia* sp.) in the city of Curitiba (Singer & Cocucci 1999) and oil in Malpighiaceae (*Heteropterys umbelata* A. Juss.) in the cerrado of São Paulo (Pedro 1994). It is well known that the oil-collecting behavior of *T. diversipes* on flowers of Malpighiaceae rarely results in contact with anthers, as the collection of this resource is held by these bees in the underside of the flowers (Rêgo & Albuquerque 1989). As described by Vogel (1990), *Tetrapedia* bees are illegitimate flower visitors on Malpighiaceae flowers. This behavior explains the low proportion of Malpighiaceae's pollen observed in the analysed samples of *T. diversipes* in this study.

In general, the diversity of sources used by *T. diversipes* during the wet season was higher in the area of eucalyptus with regenerating understory than in the secondary forest, even though the frequency of non-dominant pollen types was very low in both areas. The proximity to open and flooded areas, as well as the presence of a great number of vines and ruderal plant species around the area of regenerating forest could have influenced this result.

None of the 49 herbaceous species collected near the *T. diversipes* nesting sites in the area of eucalyptus with regenerating understory was found in the pollen samples from larval food analyzed. Among the tree-shrub species inventoried in this environment by Evaristo (2008), only the types *Byrsonima*, *Myrcia* and *Eugenia* were found, although with a low frequency (less than 15%). This result suggests that these plants are not been used as primary pollen sources, but oil and nectar. The low frequency of these types may also have resulted from accidental contact with pollen grains from these plants during foraging for other resources. These results reinforce the hypothesis that the area of eucalyptus with regenerating understory at Rebio União is being used by *T. diversipes* principally as a nesting site since the potential resources in this habitat are insufficient for maintaining the *T. diversipes* population. Thus, this study has proved that the area of eucalyptus with regenerating understory at Rebio União already does not contain preferred resources for these bees. The management of these areas through the removal of eucalyptus, currently underway, should improve the regeneration of plant species and the habitat diversity. This process is essential for increasing the availability of other important resources to the bees in the União Biological Reserve. Therefore, the monitoring of these bees is necessary to assess possible changes in the community and also to identify plants resources for the bees, including oil plants, which can colonize these areas in later successional stages.

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