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Original article

Nesting biology of *Tetrapedia amplitarsis* (Hymenoptera: Apidae: Tetrapediini)

Patrícia dos SANTOS VILHENA[1], Carolina Mayumi HIROTSU[1], and
Carlos Alberto GARÓFALO[1]

[1] Universidade de São Paulo, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Departamento de Biologia, Av. Bandeirantes, 3900, Ribeirão Preto, São Paulo, Brazil 14040-901

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Abstract – *Tetrapedia amplitarsis* is an oil-collecting solitary bee that nests in pre-existing cavities and differs from the other species of the genus in several biological aspects, as nest architecture and phenology. The aim of this study is to present detailed information on its nesting biology. Monthly field sampling was conducted from December 2011 to November 2013 using trap-nests in the Serra do Japi, Jundiaí, São Paulo, Brazil. Thirty nests were sampled, in which 168 brood cells were built. The nests consisted of molded cells in urn shape, made with a mixture of sandy material and floral oil, in linear series with horizontal orientation. Completed nests had one to 14 brood cells. Most nesting activities occurred in the hot/wet season, and the offspring emerged in the cold/dry season. The offspring time development ranged from 24 to 316 days, suggesting a univoltine life cycle but with the possibility of the occurrence of a second generation. The sex ratio was significantly female-biased (2.62♀:1♂). Unknown causes and fratricide were the main factors of mortality. *Dermestes* sp, microlepidoptera, and *Anthrax oedipus* were the natural enemies associated with the nests. Our results highlight a different *Tetrapedia* bee, for which many biological aspects are yet to be studied.

solitary bee / trap-nest / oil-collecting bee / malpighiaceae / fratricide

1. INTRODUCTION

Bees of the genus *Tetrapedia* Klug have solitary living habits, which means they build their nests alone, leaving them or dying before the offspring emerge (Michener 2007). They nest in pre-existing cavities, such as holes in tree trunks or branches, wooden buildings, or nests abandoned by other insects (Michener and Lange 1958; Camillo et al. 1995). Immatures develop inside brood cells and do not spin cocoons. Males form daily aggregations and spend the night hanging under dry branches of bushes near the nest, while females sleep inside their nests or in other pre-existing cavities (Alves-dos-Santos et al. 2002; Camillo 2005). Females collect floral oils, which are used in larval food (pollen and oil mixture) or nest construction (sand and oil) and, therefore, have a close association with oil-producing plants, especially those of the family Malpighiaceae (Menezes et al. 2012; Neves et al. 2014; Rocha-Filho and Garófalo 2016; Campos et al. 2018). These bees are pollinators of many plant species that they visit to collect nectar and pollen, including crops of economic importance, such as tomatoes (*Solanum lycopersicum* L.) (Wolowski et al. 2020).

Currently, 28 species of *Tetrapedia* are described for the Neotropical Region, 21 of which occur in Brazil (Moure and Melo 2023).

Corresponding author: P. dos Santos Vilhena, vilhena.p.s@gmail.com
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Information on nesting biology is available for only five of these species: *Tetrapedia curvitarsis* Friese, *Tetrapedia diversipes* Klug, *Tetrapedia garofaloi* Moure, *Tetrapedia rugulosa* Friese, and *Tetrapedia maura* Cresson (Michener and Lange 1958; Alves-dos-Santos et al. 2002; Camillo 2005; Rocha-Filho and Garófalo 2016). The first four species share the same nest architecture, consisting of a series of linear cells separated by partitions made of sand and floral oil (Alves-dos-Santos et al. 2002). The partition separating two cells serves as a closing plug for one of them and simultaneously as a bottom for the subsequent cell. Besides brood cells, the nests may have empty intercalary (between brood cells) and vestibular cells (before the nest closure) (Krombein 1967). Laterally, the cells are not lined with sandy material, and there is only the surface of the cavity. The closure of the nest is made of the same material as that of the partitions (Alves-dos-Santos et al. 2002).

Tetrapedia amplitarsis Friese has been recorded only in Brazil, in the states of São Paulo, Bahia, Paraná, and Mato Grosso do Sul. Most of these occurrences were documented in studies on the interactions between bees and floral resources in areas of *Cerrado* (Brazilian savannah) (Campos 1989; Pedro 1994; Andena et al. 2005; Almeida and Laroca 2013; Aguiar et al. 2017) and mixed rainforest (Weiss 2008), and identifying orchid pollinators (Cabral 2014; Pansarin et al. 2017). Although that species had been recorded in studies using trap-nests by Camillo et al. (1995) and Montagnana et al. (2021), nothing was reported on the architecture of its nests and biology, which differ in several aspects from those known for the other species of the genus.

This study aimed to gather information on various life history traits of *T. amplitarsis*, presenting data on the architecture of their nests, phenological patterns associated with the nesting season and emergence of the offspring, the developmental period of immatures, natural enemies associated with the nests, and the sex ratio of the analyzed population. Additionally, a list of plant species for which that species has been reported as pollinator or floral visitor is presented. Studies with such objectives provide crucial information for improving pollinator management and enhancing knowledge about providing adequate nesting resources for bees, as well as they contribute significantly to conserving these essential bee species (Orr et al. 2022).

2. MATERIAL AND METHODS

2.1. Study area

This study was conducted in the Reserva Biológica Municipal da Serra do Japi, in the municipality of Jundiaí, state of São Paulo, Brazil (23° 13′ 52.31"S 46° 56′ 11.88"W). This is a protected area in the center of the mountain massif of the Serra do Japi, with altitudes ranging from 700 to 1300 m above sea level. The vegetation comprises seasonal semideciduous forests, altitudinal semideciduous forests, and rocky outcrops within the Atlantic Forest domain. The morphoclimatic domain is Tropical-Atlantic, with annual rainfall reaching 1500 mm (Morellato 1992).

During the study, the climatic data of the area [monthly average air temperature (°C) and accumulated rainfall (mm)] were obtained from the climatic station of the Centro Integrado de Informações Agrometeorológicas (CIIAGRO) located in Jundiaí. Average air temperature ranged from 16.7 to 24.8 °C, respectively, in July 2013 and December 2012. October to April was the hottest period, and May to August was the coldest. Rainfall ranged from 0 to 399.2 mm, registered respectively in August 2012 and January 2012, totaling 3105.4 mm for the studied period. December to February was the rainiest period, while July to September was the least rainy. Henceforth, the period from October to April is called the "hot/wet season," and from May to September is called the "cold/dry season".

2.2. Sampling

Field sampling took place from December 2011 to November 2013 at 30-day intervals. Three sampling sites were established along a trail leading to the lookout point of the area, about 1 km apart from each other. Sampling Site 1 (S1) was located next to the administrative basis of the Reserva, under a shaded wooden hut protected from sunlight and rain. Sampling Site 2 (S2) was about 50 m inside the forest from the trail and was the most shaded site. Sampling Site 3 (S3) was at the forest's edge with more sunlight incidence.

Each site had an iron support approximately 1.7 m high covered with polypropene tile and containing trap-nests made of black cardboard tubes and bamboo canes (*Bambusa vulgaris* Schrad. ex Wendl.). The bamboo canes were cut so that a nodal septum closed one end of the cane. They had dimensions of 15 cm in length and 0.5 to 2.5 cm in diameter. The cardboard tubes had one end closed with the same material and dimensions of 6.0 cm in length and 0.6 cm in diameter (Camillo et al. 1995). Three 100 ml PVC pipes filled with bamboo of randomly selected diameters, plus two wooden plates measuring 30 × 11.5 × 4.5 cm with holes drilled to house 55 cardboard tubes each, were placed in each iron support.

During the field sampling, the trap-nests were inspected with a flashlight. Completed nests with closing plugs were removed, identified, and replaced with empty trap-nests of the same diameter. Nests built in cardboard tubes were placed inside test tubes 10 cm in length and 1.2 cm in diameter, the opening of which was closed with masking tape. The entrance hole of the bamboo canes containing nests was inserted into 10 cm long transparent polyethylene tubes, glued with masking tape, and the other end was closed with a cork.

The nests were taken to the Laboratório de Ecologia e Evolução de Abelhas e Vespas (LEEAV) of Faculdade de Filosofia Ciências e Letras de Ribeirão Preto (FFCLRP) of the Universidade de São Paulo (USP), kept in ambient room conditions, and observed daily until emergence of the adults. As adults emerged, they were captured, sacrificed by freezing at -20 °C, measured, mounted, identified, and deposited at the Coleção de Abelhas e Vespas Solitárias (CAVS) of LEEAV. A few days after the last emergence from each nest, the nests were opened, their contents analyzed, and the brood cells were counted and measured. All the sampled nests were opened and analyzed under stereoscope, including those from which no adult emerged. Bite marks on dead individuals were used to identify mortality due to fratricide. Adults dead inside the nests without molting coat were identified as having mortality due to inability to leave the nest.

2.3. Data analysis

Arithmetic Mean $\left(\overline{X}\right)$ and Standard Error (±) were used to analyze the variation in the number of brood cells per nest, the length and width of brood cells, and the number of males and females produced per nest. Repeated-measures analysis of Variance (ANOVA - *F*) was used to examine the effect of trap-nest diameter on the number of brood cells and differences in the number of nests and brood cells among sampling sites. The Kruskal-Wallis Test (*H*) was used to analyze differences in the number of brood cells with mortality among the sampling sites. Student *t*-Test (*t*) was used to analyze the significance of the differences in the number of nests and offspring emergences between the hot/wet and cold/dry seasons.

The Chi-Square Test (X^2) was used to analyze whether the sex ratio of the offspring differed from a 1:1 proportion, considering the total frequency of males and females produced and the frequency of sexes per nesting month. The development time of the immatures was regarded as the time elapsed between the date on which the nest was collected in the field and the date of the adult emergence. Spearman's Correlation Coefficients (*rs*) were calculated to determine whether the number of brood cells was correlated with cavity diameter, and whether the monthly number of brood cells built was correlated with monthly averages of air temperature (°C) and accumulated rainfall (mm).

2.4. Floral resource

A list of the plant species for which *T. amplitarsis* has been recorded as pollinator or floral visitors was made from the literature review.

3. RESULTS

3.1. Nest architecture

The space occupied by the nest within the cavity was filled with a large quantity of a mixture of sandy material and floral oil (Figure 1a). The brood cells were molded in an urn shape, with a rounded bottom and the cell closure truncated. The inner surface of the cells was lined with an additional thin layer of floral oil, making it appear smooth and shiny, and the cells were built in a linear series with a horizontal orientation (Figure 1b). The length of the cells ranged from 09 to 12 mm ($\overline{X} = 11.1 \pm 0.9$, n = 168), and the width ranged from 05 to 10 mm ($\overline{X} = 7.6 \pm 1.1$, n = 168). No intercalary or vestibular cells were observed, and no space was found between the last cell and nest plug, which was of the same material and appearance as the brood cell closures.

3.2. Nesting phenology

Thirty nests were sampled, one was built in a cardboard tube, and 29 were built in bamboo canes with inner diameters ranging from 06 to 14 mm. One hundred sixty-eight brood cells were made and distributed among the nests sampled. The number of brood cells per nest ranged from 01 to 14 ($\overline{X} = 5.53 \pm 0.62$, n = 30) and was positively correlated with the cavity diameter ($rs = 0.40$, $p = 0.029$). Higher frequencies were found in trap-nests with diameters ranging from 08 to 14 mm (Figure 2). No significant difference was found in the number of brood cells (S1: n = 64, S2: n = 51, S3: n = 53; $F = 1.226$, d.f. = 3, $p = 0.303$) or total number of nests built (S1: n = 11, S2: n = 9, S3: n = 10; $F = 2.501$, d.f. = 3, $p = 0.063$) among the three sampling sites.

In the first year (December 2011 to November 2012), nesting activities occurred exclusively in the hot/wet season (n = 40 brood cells). In the second year (December 2012 to November 2013), in addition to the 95 built cells in the hot/wet season, the bees also nested in May 2013 (n = 33 brood cells), the first month of the cold/dry season (Figure 3). The number of brood cells built in each season was significantly higher in the hot/wet season ($t = 12.63$, $p < 0.001$). Significant positive correlations were found between

Figure 1. Nests of *Tetrapedia amplitarsis* sampled in Serra do Japi, SP, Brazil, from December 2011 to November 2013. **A** Closed nest; **B** Opened nest showing brood cells and developing immatures.

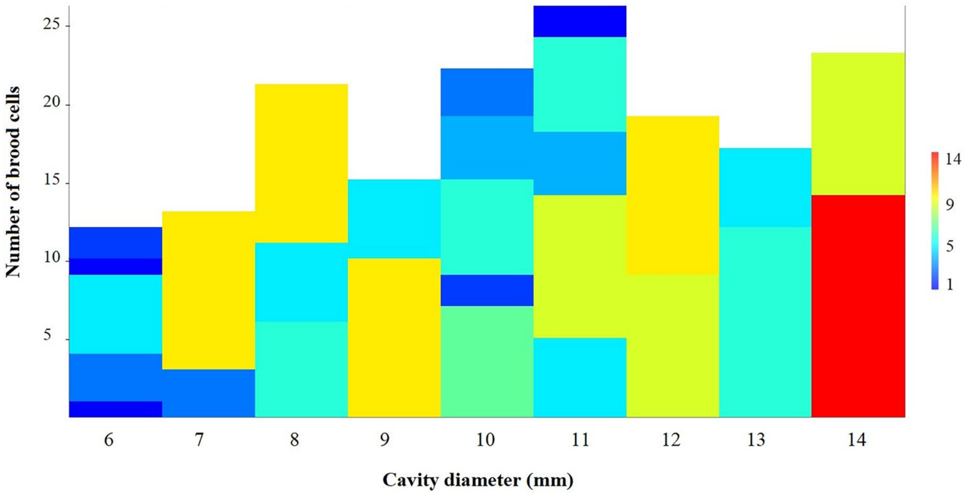

Figure 2. Number of brood cells per diameter of trap-nests occupied by *Tetrapedia amplitarsis* in Serra do Japi, SP, Brazil, from December 2011 to November 2013. Scale of cold to hot colors indicates the number of brood cells per nest in each diameter.

the number of brood cells built in each sampling month and both monthly accumulated rainfall ($rs = 0.53$, $p < 0.01$) and air temperature average ($rs = 0.43$, $p = 0.045$).

On the other hand, the number of emerged individuals was significantly higher in the cold/dry season (n = 65 individuals) than in the hot/wet season (n = 36) ($t = 3.66$, $p < 0.001$). In the hot/wet season, emergences occurred in January and April 2012 and November and December 2013 (Figure 3). Individuals produced in the first year had a development time ranging from 24 to

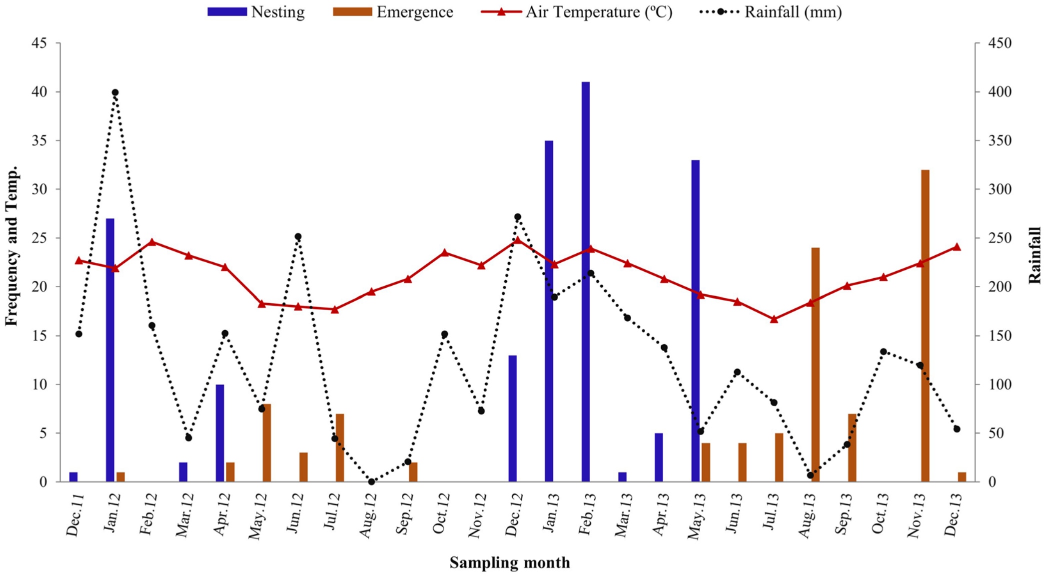

Figure 3. Nesting (number of brood cells) and emergence (number of individuals) of *Tetrapedia amplitarsis* in Serra do Japi, SP, Brazil, from December 2011 to December 2013.

173 days ($\overline{X} = 113.6 \pm 6.5$), while those emerging from nests established during the second year ranged from 84 to 316 days ($\overline{X} = 198.7 \pm 9.3$). These data suggest an univoltine life cycle, but the occurrence of some nests with immatures not undergoing diapause indicates the possibility of a second generation.

3.3. Mortality and natural enemies

Adult females and males of *T. amplitarsis* emerged from 101 (60.1%) of the 168 brood cells built in 30 nests. Among the remaining cells, mortality due to unknown causes occurred in 30 cells (17.8%), fratricide occurred in 23 cells (13.7%), mortality due to the inability to leave the nest occurred in seven cells (4.2%), and natural enemies attacked the immatures from seven brood cells (4.2%). The mortality rates due to unknown causes and fratricide were significantly similar ($\chi^2 = 0.924$, d.f. = 1, $p > 0.05$).

Mortality due to unknown causes occurred in the stages of egg (n = 9), larva (n = 9), pupa (n = 9), and adults (n = 3) distributed across eight, seven, three, and two nests, respectively. Significant differences were found in total mortality due to unknown causes among the sampling sites (S1: n = 6, S2: n = 2, S3: n = 22; $H = 6.45$, $p = 0.03$). Thirteen larvae, two pupae, and eight adults distributed across eight, two, and five nests, respectively, were victims of fratricide by adults who emerged first. Seven adults died inside the nest due to the inability to reach the exit in five nests.

The natural enemies that attacked the nests included *Anthrax oedipus* Fabricius (Diptera: Bombyliidae), *Dermestes* sp (Coleoptera: Dermestidae), and microlepidoptera (Lepidoptera). Microlepidoptera attacked four cells in the same nest. However, only cocoons were found in the brood cells, making species identification difficult. *Dermestes* sp attacked one cell from one nest, and *A. oedipus* attacked two cells, each one from one nest. All enemies had development time synchronized with those of the host. *Dermestes* sp, for example, attacked the host nest in February 2013 and took 15 months to emerge (May 2014), thus exhibiting a long development time. On the other hand, microlepidoptera and *A. oedipus* attacked nests in May 2013, and the offspring emerged in August 2013, showing short development time.

3.4. Sex ratio

The sex ratio of the offspring was significantly biased towards females, as 76 females and 29 males were recorded, resulting in a ratio of 2.62♀:1♂ ($X^2 = 39.5$, d.f.: 28, $p = 0.04$). The average number of females produced per nest was $\overline{X} = 2.53 \pm 0.33$, while the average number of males was $\overline{X} = 0.97 \pm 0.24$. The sex ratio was biased towards females in all diameters of bamboo occupied (06 to 14 mm) ($X^2 = 5.84$, d.f.: 1, $p = 0.015$) as well as when each nesting month was analyzed separately ($X^2 = 3.63$, d.f.: 1, $p = 0.021$).

3.5. Floral resources

Pollen, nectar, and floral oil are the plant resources used by *T. amplitarsis* females to build the nest and supply the cells. Females of this species have been registered visiting about 15 species from eight botanical families (Table I). Most plant species belong to the family Malpighiaceae, from which the bees collect pollen and oil. Orchidaceae is another botanical family that is relevant as an oil source. In the Serra do Japi, females of *T. amplitarsis* are the only pollinators reported for *Phymatidium delicatulum* Lindl. (Orchidaceae) (Cabral 2014). Plant species of the families Apocynaceae, Asteraceae, Fabaceae, Melastomataceae, Rubiaceae and Sterculiaceae are visited to collect pollen and nectar (Table I).

4. DISCUSSION

The nest architecture of *T. amplitarsis* differs significantly from that known for *T. diversipes*, *T. curvitarsis*, *T. garofaloi*, *T. rugulosa* (Alves-dos-Santos et al. 2002; Camillo 2005), *T. pyramidalis*

Table I Plant species for which *Tetrapedia amplitarsis* was recorded as pollinator* or floral visitor

Family	Species	Floral resource	Reference
Apocynaceae	*Forsterona glabrescens* Mull. Arg	P, N	Andena et al. (2005)
Asteraceae	*Gochnatia polimorfa* (lers.) Cabr	P, N	Andena et al. (2005)
	Bidens pilosa L	P, N	Weiss (2008)
Fabaceae	*Acosmium dasycarpum* (Vog.) Yakovl	P, N	Andena et al. (2005)
Malpighiaceae	*Banisteriopsis muricata* (Cav.) Cuatrec	P, O	Weiss (2008)
	Banisteriopsis sp.	P, O	Campos (1989)
	Byrsonima coccolobifolia A. Juss	P, O	Campos (1989), Andena et al. (2005)
	Byrsonima cydoniifolia A. Juss	P, O	Aguiar et al (2017)
	Byrsonima intermedia A. Juss	P, O	Campos (1989), Pedro (1994), Andena et al. (2005)
	Byrsonima sericea DC	P, O	Aguiar et al. (2017)
Melastomataceae	*Miconia rubiginosa* (Bonpl.) DC	P	Campos (1989)
Orchidaceae	**Phymatidium delicatulum* Lindl	O	Cabral (2014)
	Gomesa cf. *blanchetii* (Rchb.f.) Chase & Williams *(=G. montana)*	O	Pansarin et al. (2017), Castro and Singer (2019)
Rubiaceae	*Psychotria* sp.	P, N	Andena et al. (2005)
Sterculiaceae	*Walteria indica* L	P, N	Andena et al. (2005)

Floral resource: *P* Pollen, *N* Nectar, *O* Oil

and *Tetrapedia clypeata* Friese (Vilhena, P.S., unpubl. data) because these species only build partitions between the nest cells and do not mold them, entirely filling the cavity with a large quantity of sandy material. Moreover, intercalary and vestibular cells may be found in the nests of those species, but were not observed in the nests of *T. amplitarsis*.

Michener and Lange (1958) reported that females of *T. maura* carried an unidentified gray material to narrow the nesting cavity. Alves-dos-Santos et al. (2002) suggested that such material would be soil, and it was on the sides of the brood cells because they were in vertical orientation. Further, nest narrowing would be due to the reuse of the nests. Since our data prove a differentiated nest architecture, and there is no possibility that our sampled nests were reused because the occupied bamboos were removed monthly and replaced with empty ones, the same type of nest architecture may occur in *T. maura* and *T. amplitarsis*.

This difference in nesting biology among species of the same genus is similar to that found for *Centris* Fabricius, in which species of the subgenus *Hemisiella,* such as *Centris vittata* Lepeletier and *Centris tarsata* Smith, fill the occupied cavity with hard sandy soil mixed with floral oil and mold the cells within this mixture (Pereira et al. 1999; Aguiar and Garófalo 2004). On the other hand, some species of the subgenus *Heterocentris*, as *Centris analis* Fabricius, do not mold cells, building only partitions (Jesus and Garófalo 2000), although sometimes may finely line the cavity (Oliveira and Schlindwein 2009).

Observing the females of *Centris* species and those of *Tetrapedia* species that build molded and partitioned cells, no differences were noticed regarding morphological structures that could explain different nest architectures. During our study, *T. amplitarsis* co-occurred with other species (*T. diversipes*, *T. curvitarsis, T. clypeata,* and *T. pyramidalis*), having the same nesting season, and subject to the same conditions of quality and availability of floral, sandy and cavity resources. As species that mold cells must have higher energy costs to build a nest than those that make only partitions or line the cavity, why they adopt such different nest architecture strategies is worth more studies.

Aguiar and Garófalo (2004) reported that *C. tarsata* can build two parallel rows of inclined brood cells as the diameter of the cavity increases. Moreover, it has a transparent cocoon adhered to the wall of the brood cell, with a double layer of membranes on the basal part where feces are trapped. Although the nests of *T. amplitarsis* are similar to that species, that behavior was not observed, even in nests built in bamboo with the largest diameters. Further, there was no cocoon production, and the feces remained loose at the bottom of the cell.

Tetrapedia amplitarsis has a documented distribution occurring from the northeastern to southern regions of Brazil, in areas of *Cerrado* (Campos 1989; Pedro 1994; Camillo et al. 1995; Andena et al. 2005; Aguiar et al. 2017; Almeida and Laroca 2013), and Atlantic Forest as mixed ombrophilus forest (Weiss 2008), seasonal semideciduous forest, and altitudinal semideciduous seasonal forest (Cabral 2014; Pansarin et al 2017; Montagnana et al. 2021; this study). The occurrence of this species in these areas with different phytophysiognomies indicates that it has great plasticity to adapt to different environments. This characteristic together with our data of non-significant differences in the number of nests and brood cells among the sampling sites, and the results obtained by Montagnana et al. (2021) sampling *T. amplitarsis* nesting in landscape with low, intermediate, and high forest cover and different levels of habitat heterogeneity reinforce the idea that this species may be habitat-generalist.

Wide variation in diameters of used trapnests was also reported for other *Tetrapedia* species (Mesquita and Augusto 2011; Cordeiro et al. 2019). Heterogeneity in the diameters used may reflect differences in the body size of the females, as the cavity selection for nesting depends mainly on their size (Krombein 1967; Budriené et al. 2004). In the case of *T. amplitarsis*, an additional factor may be the need to mold cells, leading to a preference for larger cavities, as reported for *C. tarsata* (Aguiar and Garófalo 2004). Moreover, nesting in cardboard tubes only occurred once, even though such tubes were available in large numbers and had diameters similar to some bamboo canes used by bees. This diameter, however, corresponded to the smallest diameter among those used, suggesting that small cavities do not provide them great productivity.

Higher nesting frequency in the hot/wet season is part of the phenological pattern reported for *T. diverspes, T. curvitarsis, T. rugulosa*, and *T. garofaloi* by Camillo (2005), who observed that these species built few nests in the cold/dry season, and the last two did not occupy any traps during this season in one of the studied habitats. Gazola and Garófalo (2009) reported that the number of *T. diversipes* nests established in the cold/dry season did not differ from that of the hot/wet season. Campos et al. (2018) reported similar results for *T. curvitarsis*. In contrast, Rocha-Filho and Garófalo (2016) recorded more *T. diversipes* nests in the cold/dry season. Differences in nesting phenology among species and within the same species in different habitats have been suggested as a regular characteristic of cavity-nesting bees, and the nesting pattern must be related to climatic conditions and temporal variations in the availability of food resources (Bartomeus et al. 2011; Osorio-Canadas et al. 2018).

Our data showed that most individuals emerged during the cold/dry season, whereas only three nests were built in traps during this season, suggesting environmental adversity for nesting activity. What happens to adults is unknown. However, they may enter into adult diapause, where individuals stay inactive and sheltered in unknown places (Pereira et al. 1999; Kostál 2006), probably under leaves or within natural cavities. Or they may enter into reproductive diapause, in which adults remain active and feeding but with considerably reduced activities and without reproducing and ovipositing (Kostál 2006; Santos et al. 2019).

In studies with trap nests, it is common that unknown causes produce a higher mortality rate of immatures than that resulting from the action of natural enemies (Pereira et al 1999; Jesus and Garófalo 2000; Camillo 2005; Cavalcante et al. 2019), as well as observed for *T. amplitarsis*. However, it is important to emphasize that besides these causes two other mortality factors, rarely cited in the literature, were identified: the first, inability to leave the nest, occurred when adults produced in brood cells at the bottom of the nest completed their development before other individuals produced in cells near the nest entrance (Figure 1b). To leave the nest, they had to break several brood cells in sequence, often dying before reaching the exit. The second, fratricide, also occurred in this process, with the adult injuring larvae, pupae, and other adults, mainly with bites, when passing through brood cells containing individuals, leading them to death.

Fratricide was reported by Tepedino and Frohlich (1984) for *Osmia texana* Cresson (Megachilidae), and these authors called it as a "fratricide strategy" favored in parsivoltine species (when the adult emergence pattern of a nest is bi- or polymodal, occurring in different years) and more related to the death of males. As we found higher female mortality, naturally due to the biased sex ratio, and no directional death was found regarding the brood cell position, we cannot assume fratricide as a strategy in *T. amplitarsis* but rather an accidental event. Therefore, both fratricide and the inability to leave the nest occurred because there was no synchrony in the time of development and emergence of individuals from some nests. This is probably due to the oviposition of different females nesting in the same place, since it is known that *Tetrapedia* females cause considerable confusion regarding the identification of their nesting cavities (Michener and Lange 1958; Camillo 2005).

Nests containing all dead immature and no indication on the mortality causes were also found in our study. In these cases, the mortality seemed to be related with the architecture of the nests. It is possible that the interior of the cells becomes warmer under elevated temperatures due to the use of oil as waterproofing and soil particle binding agent, contributing to the death of the immatures. This is reinforced by the significant differences in the mortality among the sampling sites, as the shaded site (S2) had the lowest mortality rate, and the most exposed to sunlight site (S3) had the highest. Thus, a trade-off related to the architecture of the nest and the survival of the offspring can be inferred, in which a very rigid structure is efficient against the invasion of natural enemies but negatively impacts offspring survival under some environmental conditions.

The efficiency of the nest architecture of *T. amplitarsis* against invasive species is corroborated by the mortality rate of this type registered in the present study and the low number of natural enemies observed. Montagnana et al. (2021) also reported no natural enemies associated with the nests of this species. Considering *T. diversipes*, for which up to 14 species of enemies have been registered attacking its nests during a same period (Rocha-Filho and Garófalo 2016), and other species with partition-type nest architecture, parasitism rates are above 29% (*T. curvitasis* = 29.1%; *T. rugulosa* = 33.4%; *T. diversipes* = 58.6%, 31.8%, 31%) (Camillo 2005; Rocha-Filho and Garófalo 2016; Cavalcante et al. 2019). Low rates were observed only for *T. pyramidalis* (11.9%) and *T. garofaloi* (8.7%) (Vilhena, P.S., unpubl. data; Camillo 2005), which have low populational density.

Female-biased offspring sex ratio in *Tetrapedia* species was reported only once for *T. diversipes* by Cavalcante et al. (2019), but in a much smaller proportion (1.97♀:1♂) than that found in the present study. In species for which information is available, male-biased production has been found for *T. diversipes* (1.9♂:1♀), *T. garofaloi* (2.8♂:1♀), and *T. rugulosa* (1.6♂:1♀). Only *T. curvitarsis* had been reported as an equal sex ratio of 1♀:1♂ (Camillo 2005).

Environments with abundant food resources and low parasite pressure enable investment in female production by some species of solitary bees (Kim 1999; Seidelmann et al. 2010). As the sex ratio of *T. amplitarsis* remained biased towards females in all aspects analyzed, combined with the fact that there was low mortality due to natural enemies, it is accepted that the sexual allocation of this species is related to the good environmental quality in the studied area. This could indicate that the species found all resources necessary for its establishment, whether floral (pollen and oil) or otherwise (sand and cavities).

Oil-producing species of orchids, such as *Phymatidium delicatulum* Lindland and *Gomesa* cf. *blanchetii* (Rchb.f.) Chase and Williams (Orchidaceae: Oncidiinae) are found in the Serra do Japi blooming from December to February and are visited by *T. amplitarsis* (Cabral 2014; Pansarin et al. 2017; Castro and Singer 2019). Although *P. delicatulum* are self-compatible, the cross-pollination performed by these bees was more efficient in the production of fruits (54%) and seeds (82.5%) than self-pollination (31.9% and 57.9%, respectively) (Cabral 2014).

According to Pansarin et al. (2017), these orchids bloom in sympatry with other species of flowers, such as *Bidens segetum* Mart. ex Colla (Asteraceae), which offer resources such as pollen and nectar, in addition to *Stigmaphyllon*, *Banisteriopsis,* and *Peixotoa parviflora* A. Juss. (Malphigiaceae), which provide pollen and oil as resources for floral visitors. Thus, the sex ratio of the *T. amplitarsis* offspring biased to female production is favorable to botanical species in the area due to the pollination service that females provide, increasing their reproduction and, consequently, fruit and seed production.

5. CONCLUSIONS

Tetrapedia amplitarsis differs from other known species of the genus due to biological aspects related to its nest architecture, phenology, and populational sex ratio. This species builds molded cells from a mixture of sandy soil and floral oil, completely filling the nesting cavity. Most nesting activities occurs in the hot/wet season, while most of the offspring emergences occurs in the cold/dry season. The offspring development time suggests an univoltine life cycle, but the occurrence of some nests with immatures not undergoing diapause indicates the possibility of a second generation. The nest architecture is efficient against attack of natural enemies, however is a trade-off related to the survival of the offspring, because due to its hard structures some immature may died within the cells before complete development or some adults may struggle to exit the nest, accidentally dying during this process or killing other immature through fratricide. The offspring sex ratio is notably skewed to females. *Tetrapedia amplitarsis* are mostly associated with plants of the family Malpighiaceae and are the only reported pollinators of a species of orchids.

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AUTHORS' CONTRIBUTIONS

PS Vilhena and CA Garófalo contributed to the study conception and design. Material preparation, field sampling, laboratory work, and observations were performed by PS Vilhena and CM Hirotsu. PS Vilhena performed the data analyses and wrote the first draft of the manuscript. All authors revised the previous versions of the manuscript, read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIAL

Not applicable.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval This study in the protected area of Serra do Japi was approved by Prefeitura do Município de Jundiaí/Secretaria Municipal de Administração, and Fundação Serra do Japi, with protocol number of collection authorization: 97/2012.

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