

## Phoretic mites (Acari: Chaetodactylidae) associated with the solitary bee *Tetrapedia diversipes* (Apidae: Tetrapediini)\*

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**Abstract** – Solitary bees of the genus *Tetrapedia* have a specific association with mites of the genus *Roubikia* (Chaetodactylidae). These mites are frequently found attached to active *Tetrapedia* bees. We quantified the number of mites on individuals of *Tetrapedia diversipes* Klug and examined the interaction between these species. Nests of *T. diversipes* were obtained from trap-nests placed in four localities in São Paulo, Brazil. The study lasted from March 2007 to February 2009. Out of a total of 650 nests with emergences, 118 were infested with mites (*Roubikia* sp.). From these nests, 176 individuals of *T. diversipes* emerged with mites on their bodies. Additionally, six individuals of *Coelioxoides waltheriae*, the specific kleptoparasitic bee to *T. diversipes*, emerged. Mites were attached mainly to the mesosoma. All nests infested with mites did not presented mortality of the immature. The mortality rate of nests was inversely related to the level of mite infestation, suggesting a mutualistic interaction in which mites may remove fungi from the nests, while the bees would provide the mites with transport, dispersal, and shelter.

solitary bee / phoresy / trap-nest / Atlantic Forest / symbiosis

### 1. INTRODUCTION

Mites and bees have co-existed since the Cretaceous, and there is evidence of a close relationship between some taxa, probably resulting from a co-evolutionary processes (Klimov et al., 2007a). Most mite lineages associated with bees use species of Apidae and Megachilidae as hosts (Eickwort, 1994; Klimov et al., 2007a). These mites belong to the family Chaetodactylidae (Acari: Astigmata). Associations with basal lineages of Megachilidae

(Lithurgini) and Apidae (Xylocopini) suggest that chaetodactylids may have established themselves in the Lower Cretaceous, when these two bee families became separated (Engel, 2001). Another hypothesis is that the mites arose in a later period, during the Eocene, when many exchanges among hosts would have occurred simultaneously with the dispersal of bees through the continents (Klimov and O'Connor, 2007).

Chaetodactylidae comprises over 200 described species within five genera and distributed worldwide, except for the poles (Klimov and O'Connor, 2008). The active initial phases of the mites (larva and nymph) have been described for 85 species. During

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these phases, mites live within the bee nests and feed on materials found in the brood cells, and may be mutualists (consuming nest wastes), parasitoids (killing the egg or the larva), commensals or parasites (consuming the larval food and so hindering the development of the larva) (Krombein, 1962; Roubik, 1987; Qu et al., 2002). Most Chaetodactylidae species are found in association with bees in the phases of deutonymph or phoretic hypopus, frequently positioned on the acarinarium (Fain, 1981). In this dispersal stage, the mite stops feeding and becomes inactive; it is transported by the host to the next site where a new cycle begins (Baker et al., 1987). The hypopi have a morphology fully adapted to this life phase: they have no mouthparts or digestive tract, a highly sclerotized carapace, which prevents dehydration, robust pretarsal claws, and caudoventral suckers for attaching to the host (Houck and O'Connor, 1991). In most cases, a certain synchronism between the life cycles of both associates occurs (Klimov and O'Connor, 2007).

Some bee species exhibit specialized regions on the body called acarinarium where mites are frequently lodged (O'Connor and Klompen, 1999). Acarinarium are small cavities, sometimes covered with setae, which facilitate the attachment of mites to the bee's body and provide them protection during transport. These structures occur in many families of aculeate hymenopterans (Eickwort, 1994; O'Connor and Klompen, 1999) and are evidence for the strong reciprocity that those interactions have been present through evolutionary time. However, not all bees with associated mites exhibit developed acarinarium, and the mites can, therefore, be distributed randomly on the host body (Klimov et al., 2007b). Among bees of the family Apidae, acarinarium are present in species of *Xylocopa*, *Ceratina*, and *Tetrapedia* (Klimov and O'Connor, 2007). However, these features not always result from mutualistic associations (Klimov et al., 2007b).

Despite the specificities within associations, host changes may occur. In some cases, they occur by mistake. For instance, a mite may remain on flowers (ex. *Sennertia*) and attach to different bees, or a mite that blends in

the sand and soil particles may attach to certain bees that collect this material (Roubik, 1987; Vicidomini, 1996). There are frequent changes between relatively close taxa within the same genus (Klimov et al., 2007b). Approximately 170 *Sennertia* species are associated with 469 species of *Xylocopa* (Michener, 2000; Klimov and O'Connor, 2007).

Among the solitary bees, the genus *Tetrapedia* (Apidae, Tetrapediini), a group of oil-collecting bees, was reported to be in specific association with mites of the genus *Roubikia* (Roubik, 1987; Klimov and O'Connor, 2007; Klimov et al., 2007a, b). The mites also lodge on *Coelioxoides* (Apidae: Tetrapediini), their specific kleptoparasitic species (Klimov et al., 2007a). It was known that the interaction between *Roubikia* and *Tetrapedia* is commensalistic: the mites feed on larval food and on nest materials without causing any harm to the immature (Klimov et al., 2007b). The association is restricted to the Neotropical region, which is an area of occurrence for both groups.

Alves-dos-Santos et al. (2002) and Camillo (2005) studied the nesting biology and the nest architecture of *Tetrapedia diversipes*. This species has a bivoltine cycle: the first generation is active in the spring, whereas the second is active in the summer. The females nest on wood substrate and frequently use pre-existing cavities in tree bark made by beetles; in such cases, they may be captured in trap-nests (Garófalo et al., 2004). Old nests are frequently re-used. The oil collected by the females is used for nest construction, compacted with sand particles, and mixed together with pollen for larval food. The larvae of *Tetrapedia* develop in cells and do not spin cocoons. *Coelioxoides waltheriae* Ducke is the specific kleptoparasitic species of *T. diversipes*.

We suppose that if the relationship of *Roubikia* mites with *T. diversipes* is mutualistic, than a lower mortality rate would be found when the mites are present. Therefore, the objectives of the present study were to verify the infestation by mites of the genus *Roubikia* both in nests and on emerging individuals of the solitary bee *Tetrapedia diversipes* and to study the interaction between them.

## 2. MATERIAL AND METHODS

We obtained nests of *Tetrapedia diversipes* by setting up trap-nests. This method is widespread for the capture of solitary bees and consists of offering artificial cavities through cardboard tubes lodged in perforated wooden blocks or bamboo tubes (Camillo et al., 1995; Garófalo et al., 2004).

The present study was carried out for two years, between March 2007 and February 2009, in four areas within the dense rainforest domain of the Atlantic Forest of the state of São Paulo, Brazil: Parque Estadual de Ilhabela (23°45'S–45°27'W, altitude: 0–1.378 m, São Sebastião municipality), Parque Estadual da Serra da Cantareira (23°30'S–46°45'W, altitude: 950–1.074 m, São Paulo municipality), Parque das Neblinas (23°45'S–46°09'W, altitude: 700–1.100 m, Mogi das Cruzes municipality), and Estação Biológica de Boracéia (23°38'S–45°52'W, altitude: 750–900 m, Salesópolis municipality). Additional nest material came from the Bee Laboratory at the campus of the University of São Paulo - USP (23°33'S–46°43'W, São Paulo municipality). In Parque Estadual da Serra da Cantareira the sampling was carried out within a year, between March 2007 and February 2008.

We inspected trap-nests monthly. Established nests were removed from the field and taken to the laboratory, where they remained lodged in individual test tubes capped with a cork. The emerging individuals were killed with ethyl acetate and prepared for mounting in an entomological collection. Finally, we opened the nests in order to study their internal contents and mortality record. We analyzed thoroughly all emerging individuals under a stereomicroscope for the selection of bees with mites on the body. We counted the mites on bees according to the place of attachment to the bee's body.

We separated three infested nests from the University's campus after the emergence of the bees in order to study their internal content. The nests were opened and placed in Petri dishes and kept in a dark environment. We followed the mites' eggs until they hatched.

Flowers of sweet basil (*Ocimum basilicum*, Lamiaceae) from the garden at the Bee Laboratory at the University, which are frequently visited by *Tetrapedia diversipes*, were collected and examined to check for the presence of mites. Bees were also collected on the flowers to check for the presence of mites and the place of attachment to the body of bees in activity.

We recorded images of bees with mites using a camera attached to a stereomicroscope (Leica -

MZ16, software IM50). For further details, images were made with a scanning electron microscope (SEM) in the laboratory NAP/MEPA at ESALQ – USP Campus Piracicaba. We glued dried specimens in stubs with a double-sided tape at the desired position and subjected them to gold metallization.

We identified the hypopi based on the study and identification keys by O'Connor (1993b) and Klimov et al. (2007b). All examined samples were deposited in the Entomological Collection Paulo Nogueira Neto (CEPANN) of the Biosciences Institute of University of São Paulo.

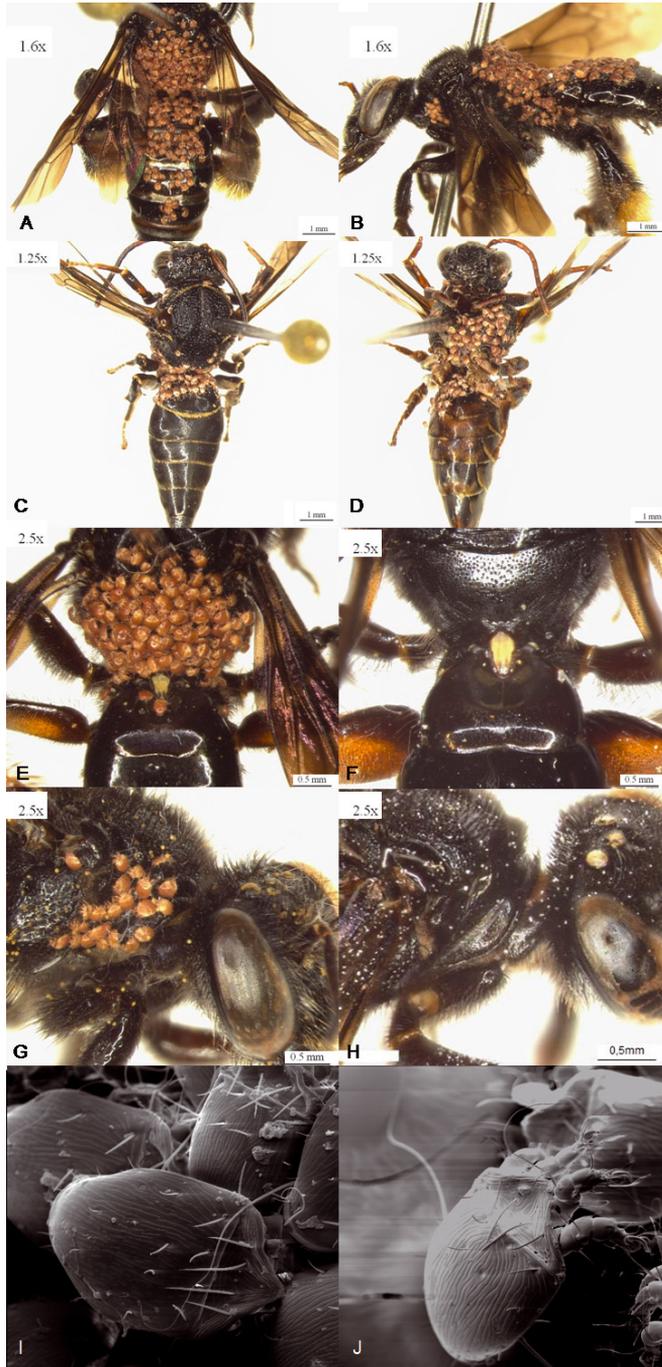
We tested whether the sex ratio deviated from the expected proportion of 1:1 using a Chi-square test. The effect of infestation on survival and the difference of infestation between sexes were tested with a t-test. We correlated the rate of infested nests by mites with the mortality rate of nests in the four areas to check the relationship between the two variables. To test the effect of seasonality in the infested nests, we used the Chi-square test. Significance was determined at the 0.05 level.

## 3. RESULTS

We obtained 913 nests of *Tetrapedia diversipes* from the traps disposed in the four areas. Among them, occupants emerged from 650 nests, from which 118 (18.1%) were infested with mites (Tab. I). In the remaining 263 nests, we recorded the total mortality of the occupants at different development phases (larva, pupa and pre-emergence adult). The main diagnosed cause for immature mortality was the infestation by fungi.

From the nests infested by mites, 176 individuals of *T. diversipes* emerged (142 males and 34 females) bearing mites on their bodies (Figs. 1A, B), which correspond to 81% of the bees in these nests. In 91% of the infested nests, all individuals carried mites. The infestation rate of mites from the total of emerging individuals was 14.3% (Tab. II).

Six individuals of *Coelioxoides waltheriae*, the specific kleptoparasitic species of *T. diversipes*, also emerged with attached mites (Figs. 1C, D), one from each infested nest. Individuals of the parasitic species *Anthrax* (Diptera) that emerged from infested nests did not have mites attached to their body.



**Figure 1.** A and B: Male of *Tetrapedia diversipes* with mites attached in dorsal view and in lateral view. C and D: *Coelioxoides waltheriae* with mites attached in dorsal view and in ventral view. E and F: view of the parts with higher concentration of mites. Region of the propodeum and scutellum with and without mites. G and H: region of the pronotum with and without mites. I and J: images in SEM of individuals of *Roubikia* sp on the mesosoma.

**Table I.** Nests founded by *Tetrapedia diversipes* in the four study areas from March 2007 to February 2009, and mite infestation in the nests with emergence.

Study areas	Founded nests	Nests with total mortality	Nests with emergence	Nests infested with mites	% of nests infested with mites
Ilhabela	501	141	360	76	21.1
Boracéia	228	50	178	36	20.2
Pque. das Neblinas	51	17	34	4	11.8
Cantareira*	133	55	78	2	2.6
Total	913	263	650	118	18.1

\* In this locality the sampling took only one year.

**Table II.** Emerging individuals and individuals of *Tetrapedia diversipes* with mites in the four study areas from March 2007 to February 2009.

Study areas	Emerging individuals	♂	♀	Individuals with mites	% ind. with mites	♂ with mites	♀ with mites
Ilhabela	650	456	194	97	14,9	85	12
Boracéia	353	179	174	64	18,1	42	22
Pque. das Neblinas	66	37	29	9	13,6	9	0
Cantareira*	159	67	92	6	3.8	6	0
Total	1228	739	489	176	14.3	142	34

\* In this locality the sampling took only one year.

In the areas of Ilhabela and Boracéia the occurrence of nests with mites was higher than in Parque das Neblinas and Cantareira (Tab. I). The infestation rate of individuals in Cantareira was the lowest (Tab. II).

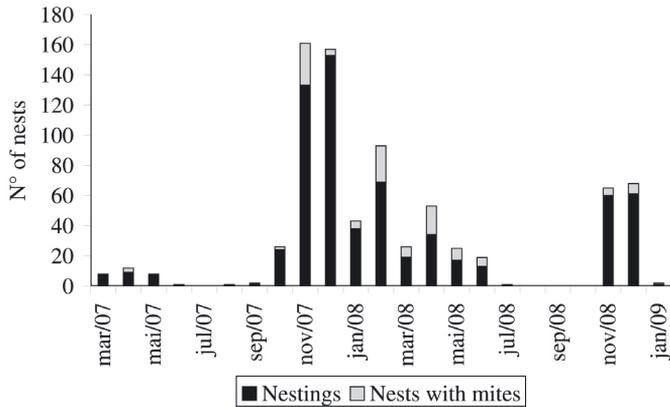
The infestation of *T. diversipes* was higher in males in all localities; however, this trend was not significant ( $t = 0.369$ ,  $df = 122$ ,  $P = 0.71$ ) (Tab. II). In Ilhabela, this pattern would be expected since the sex ratio in this locality is clearly biased towards males ( $\chi^2 = 105.606$ ,  $P = 8.99 \times 10^{-25}$ ). However, in the localities where the sex ratio was not significantly biased (Boracéia  $\chi^2 = 0.0708$ ,  $P = 0.79$  and Neblinas  $\chi^2 = 0.969$ ,  $P = 0.325$ ), or even where it was biased towards females (Cantareira  $\chi^2 = 3.93$ ,  $P = 0.04$ ), the proportion of infected males was still higher.

Throughout the studied period the highest infestation index of mites in nests coincided with the highest nesting rate, which was spring and summer (mainly November 2007 and February 2008); peaks probably correspond to the two annual generations of *T. diversipes* (Fig. 2). The number of nests with

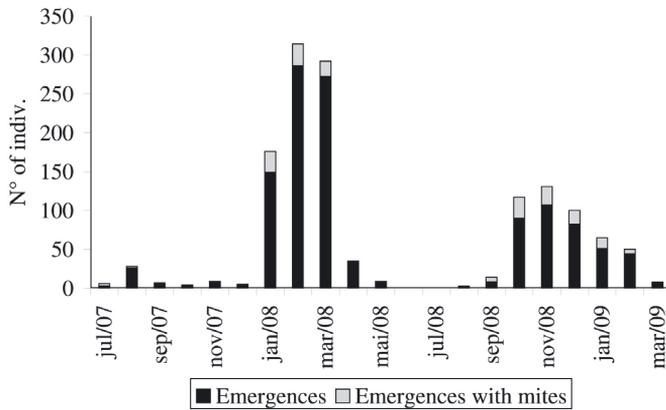
mites shows high seasonality ( $\chi^2 = 421.9$ ,  $P = 0.00001$ ). Likewise, the proportion of emerging individuals with mites was higher during those seasons (Fig. 3).

There was an average of  $77.8 \pm 69.10$  mites per individual ( $n = 97$ ) bee. However, we observed specimens carrying a maximum of 385 mites and others with only two individuals (Tab. III). The favorite area for mite attachment was the dorsal mesosoma, mainly on the scutellum, propodeum and pronotum (Figs. 1A, E, 4). However, we also found mites attached to other parts of the bees' body, such as the mesepisternum, the metasoma (terga and sterna) and the legs. Figures 1I, J show the hypopus of *Roubikia* in detail. In *Coeioxoides waltheriae*, the main area of attachment also corresponds to the region around the petiole (scutellum, propodeum and abdominal tergum) as well as the ventral mesosoma (Figs. 1C, D).

The mortality in nests showed a clear trend of a negative correlation with the infestation by *Roubikia* (Tab. I, Fig. 5). In all infested nests



**Figure 2.** Nestings of *Tetrapedia diversipes* and nests with mites between March 2007 and February 2009 in the four study areas.

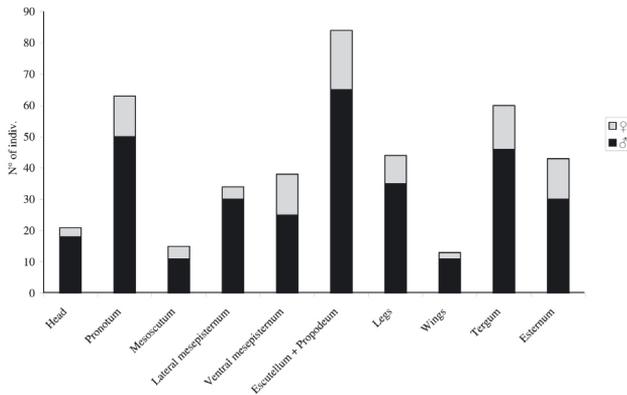


**Figure 3.** Emerging individuals and individuals of *Tetrapedia diversipes* with mites between March 2007 and February 2009.

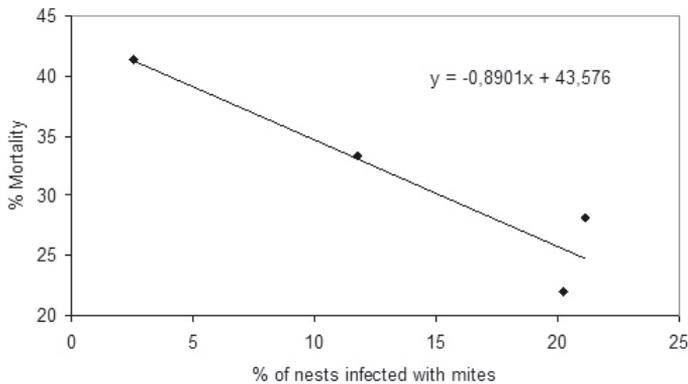
**Table III.** Number of mites on individuals of *Tetrapedia diversipes* in the four study areas from March 2007 to February 2009.

Study areas	Mites per individual average $\pm$ std. dev. (min–max)	N° individuals examined (N)
Ilhabela	47.4 $\pm$ 70.25 (2–385)	53
Boracéia	132.3 $\pm$ 58.36 (5–270)	27
Pque. das Neblinas	98.7 $\pm$ 72.58 (15–177)	9
Cantareira*	72.6 $\pm$ 64.14 (22–234)	8
Total	77.8 $\pm$ 69.10 (2–385)	97

\* In this locality the sampling took only one year.



**Figure 4.** Number of male and female individuals of *Tetrapedia diversipes* examined ( $n = 97$ ) with mites attached to different parts of the body.



**Figure 5.** Relationship between total mortality in the nests of *Tetrapedia diversipes* and the percentage of mite infestation in the nests.

we did not record mortality, that is to say, the mites did not caused the death of individuals of *T. diversipes*. On the other hand, in uninfested nests, the mortality was about 15%. This positive effect of infestation in the nests of *T. diversipes* with mites was significant ( $t = 5.03$ ;  $df = 644$ ;  $P < 0.0001$ ).

On the examined sweet basil flowers we found no mites; however, some females collected while visiting the flowers had mites attached to their bodies, mainly on the sides of the mesepisternum and some on the terga.

In the infested nests kept in Petri dishes, we observed several development phases of mites. Larvae and nymphs were observed every day moving around and among a pollen

mass mixture with many exuviae, sand particles and feces of the host larvae inside the nest. Only a few hypopi were observed. We detected the presence of eggs from the first to the sixth day, and we observed a higher amount during the first three days. The eggs were tiny (about 0.1 mm), elliptical and with a white-ish-translucent appearance when first laid; from the second day on, they exhibited an opaque color and a granular texture. From the third day on, some larvae started emerging.

#### 4. DISCUSSION

Previous studies carried out on the associations between *Roubikia* mites and *Tetrapedia*

bees (Baker et al., 1987; Roubik, 1987) showed that this is a case of commensalism. Mites apparently ingest wastes of larval food and nest materials without causing any harm to the immatures (Klimov et al., 2007a). Roubik (1987) proposed that the mites may also consume, in addition to the pollen, the flower oils present in the stock. The presence of a large amount of nymphs moving about the pollen mass in the nests opened for observation suggests that these nymphs may be feeding on wastes of the food previously consumed by bee larvae. Another evidence for this hypothesis is the presence of many exuviae with the pollen mass. Additionally other materials in the nests could also be consumed, such as larval feces, which were loose inside the cells, parts of exuviae of the bee larvae, and developing fungi.

Our results showed that the mortality in nests has a tendency of a negative correlation with the infestation by *Roubikia* (Fig. 5). In infested nests, no fungus was found and there was no mortality, suggesting that bees benefit from the presence of mites. Flechtmann and Camargo (1974) observed a mutualistic relationship of *Neotydeolus* mites (Tydeidae) in colonies of *Scaptotrigona postica* (Apidae), where the proliferation of fungi caused the death of brood. After the introduction of mites in the colonies, the propagation of fungi decreased rapidly, likely reducing death in the brood comb. Furthermore, O'Connor (1979) recorded fungivorous ancestors for mites of Astigmata. Recently, Biani et al. (2009) showed the mutualistic role of *Laelaspoides* mites (Laelapidae) in nests of *Megalopta* (Halictidae), where the mites eliminate the fungi that proliferate in nests.

Studies with solitary bees demonstrate the presence of fungi in the nests as the main cause of death (Morato, 2001; Camarotti-de-Lima and Martins, 2005). Although *T. diversipes* is a widespread species, the studied nests are from areas of Atlantic Forest with high rainfall rates, in particular during the summer, which most likely contributes to the proliferation of fungi. Possibly, *Roubikia* mites are eliminating fungi in *T. diversipes* nests since no bee died in the infested nests.

Studies carried out with species of *Chaetodactylus* (Chaetodactylidae) in *Osmia cornifrons* (Megachilidae) reported that the type of interaction may vary with the density of mites; in small quantities, the interaction is commensal, whereas in large quantities (>50 mites per cell), it becomes negative (Qu et al., 2002). This pattern is frequently observed in social bees (ex. *Varroa* in *Apis mellifera*), on which the high infestation rate in brood combs may lead to the collapse of the colony (Oldroyd, 1999; Menezes et al., 2009). In our study, even the bees that carried over 300 mites on their body emerged, i.e., even in large quantities the mites did not represent a death risk for *T. diversipes* individuals.

The parts of the body of *T. diversipes* with the highest concentration of mites (pronotum, scutellum and propodeum) are not characterized as acarinarium but as mesosoma depressions (Figs. 1E–H); here, the mites remain less exposed and less vulnerable during the transport. The attachment to other parts of the body, such as the spaces between the terga, the ventral region between the coxae or the sternum setae, probably occur for the same reason. In the observed bees, this generalized distribution occurred in individuals with a high amount of mites on the mesosoma, and the mites probably spread due to the intense agglomeration. Additionally, probably those regions of the body are difficult to reach during self-grooming. According to Krombein (1962), the mites of Chaetodactylidae may attach to the body of bees with claws or suckers that are ventrally located. In *T. diversipes*, in the mesosoma depressions lacking many setae (pronotum, scutellum, propodeum, terga and ventral mesepisternum), the mites should attach mainly with suckers; and in the regions full of setae (sterna, lateral mesepisternum and legs), they should attach with claws. Klimov et al. (2007a) observed in Peru an incipient acarinarium in a species of *Tetrapedia* located in the lowest part of the first metasomal tergum infested with mites of *Roubikia latebrosa*. However, they did not find this structure in any other species of *Tetrapedia* examined in the study.

We observed all the development phases of the mite – eggs, larvae, and nymphs, including

some hypopi – simultaneously within the nests, where the bees had already emerged. Trouessart (1904) observed the occurrence of two types of hypopi in *Chaetodactylus osmiae*, a species associated with *Osmia cornuta* (Apidae): a phoretic form and an encysted one (Fain, 1966). According to the previous author, the phoretic form has a dispersal function, whereas the encysted form, present in higher numbers, remains in the nest in a quiescent state. The hypopi observed in the nests of *T. diversipes* are probably encysted, waiting for another host female, in case the nest is re-used. This strategy is adopted by several mite species (Krombein, 1962). While environmental conditions are favorable and there is food enough in the nest, the mites remain there to develop and reproduce. The transformation of protonymphs into hypopi occurs in different ways among mite species, but it usually occurs due to limiting factors such as lack of food, change in environmental conditions (extreme temperatures or low humidity), or it is activated according to the maturation of the host into adults. This synchrony is possibly caused by chemical substances (secretions and hormones) released by the host and detected by mites (Houck and O'Connor, 1991). The mites of *Roubikia* observed in the present study attach themselves only to bees, both the hosts (*T. diversipes*) and the kleptoparasites (*C. waltherriae*), and not to parasitic dipterans (*Anthrax*), possibly due to chemical differences detected by mites too.

The hypopus phase is characterized by extreme resistance, and it may take days, weeks and even months (Houck and O'Connor, 1991). Schwarz and Huck (1997) recorded some mite species in which the hypopus remained for about 24 h on flowers, waiting for *Bombus* bees (Hymenoptera: Apidae) to disperse it among nests. We found that both routes of development may occur in *Roubikia*. The mites continue the cycle while there is food available and may infest the next brood in case of reutilization of the nest, and others turn into hypopi and ascend the bee about to emerge or remain encysted in the nest. Unlike Trouessart (1904), it was observed a higher amount of phoretic hypopi in relation to encysted ones.

In accordance with studies on other mites of Chaetodactylidae (Krombein, 1962; Park et al., 2009), the dispersal of the hypopi attached to the female host occurs when it provides the cells with the collected material (pollen, nectar or oil). The hypopi then step down to the new nest, undergo a molt to the adult stage and reproduce, starting the new generation (Qu et al., 2002). Females of *Tetrapedia* that were visiting sweet basil flowers (*Ocimum basilicum*) exhibited mites attached mainly to the sides of the mesepisternum, and some attached to the terga, differently from newly emerged bees that had mites dorsally concentrated, on the scutellum and propodeum. This distribution corroborates the dispersal behavior of the hypopi previously mentioned: since the females are building nests, the mites could be already positioned to descend to the nest. Another possibility would be that they concentrate on these parts to descend to flowers, or because they had shifted from flowers to bees, corroborating the hypothesis of the dispersal through flowers. However, we must keep in mind that the flowers visited by *T. diversipes* in the garden of the University were examined, and mites were not detected.

Furthermore, we must take into account that the infestation rate was higher in males of *T. diversipes* in all localities, even when there was a bias in the sex ratio towards females (Cantareira). In this case, a possible strategy could be the contamination or dispersal among the same nests. The nests of *T. diversipes* captured in trap-nests are clustered, located a few centimeters apart from each other. The most external cells are usually constructed for males (Alves dos Santos et al., 2002). Krombein (1962) also recorded higher infestation by *Chaetodactylus krombeini* in males of *Osmia lignaria* (Megachilidae). For the mites, the infestation of male bees may represent a broader range of dispersal, since they can fly longer distances searching for females. Regardless, the mechanisms of the mites dispersal remain unclear. According to Park et al. (2009) the mites carried by males would be impaired unless they were transferred to new females during the copula. Further studies are necessary to investigate how *Roubikia* mites disperse and if

and how they provide a mutualistic benefit to *Tetrapedia* bees.

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**Acariens phorétiques (Acari : Chaetodactylidae) associés à l'abeille solitaire *Tetrapedia diversipes* (Apidae : Tetrapediini).**

**Abeille solitaire / phorésie / nid-piège / Forêt atlantique / symbiose / Brésil / *Roubikia* / *Coelioxoides***

**Zusammenfassung – Phoretische Milben (Acari: Chaetodactylidae), die mit der solitären Biene *Tetrapedia diversipes* (Apidae: Tetrapediini) assoziiert sind.** Wir quantifizierten die Zahl der Milben auf Bienen der Art *Tetrapedia diversipes* und untersuchten deren Interaktionen. Die Studie wurde im Zeitraum März 2007 bis Februar 2009 im Atlantischen Regenwald des Staats São Paulo, Brasilien an vier Standorten durchgeführt, an der Biologischen Station Boracéia, im Neblina Park, sowie in den Schutzgebieten Ilhabela und Serra da Cantareira. Mittels Nistfallen unterschiedlichen Durchmessers war es möglich, solitäre Bienen zu finden und deren Nester dann ins Labor zu bringen. Alle schlüpfenden Bienen wurden mikroskopisch auf Milben hin untersucht und letztere wurden nach Fundorten auf den Bienen getrennt gezählt. Aus 650 der insgesamt 913 Nestern, die an den vier Standorten gesammelt worden waren, schlüpfen Bienen, wobei Bienen aus 118 (18,1%) der Nester mit Milben infiziert waren. In den verbleibenden 263 Nestern konnten wir als vermutlichen Grund der Mortalität Pilzinfektionen ausmachen. Aus den Nestern mit Milbenbefall schlüpfen 176 Bienen,

die Milben an ihrem Körper trugen, was 81 % der Bienen aus diesen Nestern entspricht. Im Bezug auf die Gesamtzahl der schlüpfenden Bienen lag der Milbenbefall bei 14,3 %. Darunter fanden wir auch sechs Individuen der Art *Coelioxoides walt-heriae*, eines spezifischen Kleptoparasiten von *T. diversipes*, die ebenfalls mit Milbenbesatz schlüpfen. An den Standorten Ilhabela und Boracéia fanden wir eine höhere Zahl an Nestern mit Milbenbefall als in den Parks Neblina und Cantareira. Im Mittel fanden wir  $77,8 \pm 69,10$  Milben pro Biene ( $n = 97$ ) und zwar bevorzugt auf der dorsalen Region des Mesosoma-Segments. Obwohl der Körper von *T. diversipes* zwar keine sogenannten Acarinaria-Regionen aufweist, stellen die Eindellungen des Mesosomas doch Orte dar, an denen die Milben weniger exponiert und damit während des Transports besser geschützt sind. Frühere Studien über die Assoziation von *Roubikia* Milben mit *Tetrapedia* Bienen hatten angedeutet, dass es sich hierbei um Kommensalismus handelt. In unserer Studie zeigten jedoch die Mortalitätsraten der Bienen in den Nestern eine negative Korrelation mit dem Milbenbefall, was darauf hinweist, dass die Bienen von den Milben profitieren. In Nestern mit Milbenbefall war kein Pilz zu finden und auch keine Mortalität, und Studien an anderen solitären Bienen hatten bereits gezeigt, dass Pilzbefall eine Hauptursache der Mortalität ist. *T. diversipes* ist eine weitverbreitete Art. Die Standorte, an denen wir sammelten lagen jedoch alle im Bereich des Atlantischen Regenwalds, dessen hohe Feuchtigkeit dem Pilzwachstum förderlich ist. Da wir in den Nestern mit Milbenbefall keine toten Bienen fanden, ist es möglich, dass *Roubikia* Milben diese Pilze in Schach halten.

**Solitärbiene / Phoresie / Nistfallen / Atlantischer Regenwald / Symbiose**

## REFERENCES

- Alves-dos-Santos I., Melo G.A.R., Rozen J.G. (2002) Biology and immature stages of the bee tribe Tetrapediini (Hymenoptera: Apidae), Am. Mus. Novitates 3377, 1–45.
- Baker E.W., Roubik D.W., Delfinado-Baker M. (1987) The developmental stages and dimorphic males of *Chaetodactylus panamensis*, n. sp. (Acari: Chaetodactylidae) associated with solitary bee (Apoidea: Anthophoridae), Int. J. Acarol. 13, 65–73.
- Biani N.B., Mueller U.G., Wcislo W.T. (2009) Cleaner mites: sanitary mutualism in the miniature ecosystem of neotropical bee nests, Am. Nat. 173, 841–847.
- Camarotti-de-Lima M.F., Martins C.F. (2005) Biologia de nidificação e aspectos ecológicos de *Anthodioctes lunatus* (Smith)

- (Hymenoptera: Megachilidae, Anthidiini) em área de tabuleiro nordestino, PB. Neotrop. Entomol. 34, 375–380.
- Camillo E. (2005) Nesting biology of four *Tetrapedia* species in trap-nests (Hymenoptera, Apidae, Tetrapediini), Rev. Biol. Trop. 53, 175–186.
- Camillo E., Garófalo C.A., Serrano J.C., Mucilo G. (1995) Diversidade e abundância sazonal de abelhas e vespas solitárias em ninhos-armadilhas (Hymenoptera, Apocrita, Aculeata), Rev. Bras. Entomol. 39, 459–470.
- Eickwort G.C. (1994) Evolution and life-history patterns of mites associated with bees, in: Houck MA (Ed.), Mites: Ecological and Evolutionary Analyses of Life-History Patterns, New York, Chapman & Hall, pp. 218–251.
- Engel M.S. (2001) A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera), Bull. Am. Mus. Nat. Hist. 259, 1–192.
- Fain A. (1966) Notes sur la biologie des acariens du genre *Chaetodactylus* et en particulier de *C. osmiae*, parasite des abeilles solitaires *Osmia rufa* et *O. cornuta* en Belgique (Sarcoptiformes: Chaetodactylidae), Bull. Ann. Soc. R. Belge Entomol. 102, 249–261.
- Fain A. (1981) Notes on the hypopi of the genus *Chaetodactylus* Rondani, 1866 (Acari, Chaetodactylidae), Bull. Inst. R. Sci. Nat. Belg. Entomol. 53, 1–9.
- Flechtmann C.H.W., Camargo C.A. (1974) Acari associated with stingless bees (Meliponidae, Hymenoptera) from Brazil, in: Piffel E. (Ed.), Proc. 4th Int. Congr. Acarol. Budapest, Académiai Kiadó, pp. 315–319.
- Garófalo C.A., Martins C.F., Alves-dos-Santos I. (2004) The Brazilian solitary bee species caught in trap nests, in: International Workshop on solitary bees and their role in pollination, Beberibe, CE. Solitary Bees: conservation, rearing and management for pollination, Fortaleza: Imprensa Universitária, pp. 77–84.
- Houck M.A., O'Connor B.M. (1991) Ecological and evolutionary significance of phoresy in the Astigmata, Annu. Rev. Entomol. 36, 611–636.
- Klimov P.B., O'Connor B.M. (2007) Ancestral area analysis of chaetodactylid mites (Acari: Chaetodactylidae), with description of new early derivative genus and six new species from the Neotropics, Ann. Entomol. Soc. Am. 100, 810–829.
- Klimov P.B., O'Connor B.M. (2008) Morphology, Evolution, and Host Associations of Bee-Associated Mites of the Family Chaetodactylidae (Acari: Astigmata), with a monographic revision of North American taxa, Miscellaneous Publications, Museum of Zoology, University of Michigan 199, pp. 1–243.
- Klimov P.B., O'Connor B.M., Knowles L.L. (2007a) Museum specimens and phylogenies elucidate ecology's role in coevolutionary associations between mites and their bee hosts, Evolution 61, 1368–1379.
- Klimov P.B., Vinson S.B., O'Connor B.M. (2007b) Acarinaria in associations of apid bees (Hymenoptera) and chaetodactylid mites (Acari), Invertebr. Syst. 21, 109–136.
- Krombein K.V. (1962) Natural history of Plummers Island, Maryland. XVI. Biological notes on *Chaetodactylus krombeini* Baker, a parasitic mite of the megachilid bee, *Osmia (Osmia) lignaria* Say (Acarina, Chaetodactylidae), Proc. Biol. Soc. Wash. 75, 237–250.
- Menezes C., Coletto-Silva A., Gazeta G.S., Kerr W.E. (2009) Infestation by *Pyemotes tritici* (Acari, Pyemotidae) causes death of stingless bee colonies (Hymenoptera: Meliponina), Genet. Mol. Res. 8, 630–634.
- Michener C.D. (2000) The Bees of the World. Baltimore, The John Hopkins University Press.
- Morato E.F. (2001) Biologia e ecologia de *Anthodictes morato* Urban (Hymenoptera, Megachilidae, Anthidiini) em matas contínuas e fragmentos na Amazônia Central, Brasil. Rev. Bras. Zool. 18, 729–736.
- O'Connor B.M. (1979) Evolutionary origins of astigmatid mites inhabiting stored products, in: Rodriguez J.G. (Ed.), Recent advances in acarology (1), Academic Press, New York, pp. 273–278.
- O'Connor B.M. (1993a) The mite community associated with *Xylocopa latipes* (Hymenoptera: Anthophoridae: Xylocopinae) with description of a new type of acarinarium, Int. J. Acarol. 19, 159–166.
- O'Connor B.M. (1993b) Generic relationships in the Chaetodactylidae (Acari: Astigmata) with description of a new genus, Acarologia 34, 345–362.
- O'Connor B.M., Klompen J.S.H. (1999) Phylogenetic perspectives on mite-insect associations: the evolution of acarinarium, in: Needham G.R., Mitchell R., Horn D.J., Welbourn W.C. (Eds.), Acarology IX, Vol. 2, Symposia. Columbus, Ohio, Ohio Biological Survey, pp. 63–71.
- Okabe K., Makino S. (2002) Phoretic mite fauna on the large carpenter bee *Xylocopa appendiculata circumvolans* (Hymenoptera: Apidae) with descriptions of its acarinarium on both sexes, J. Acarol. Soc. Jpn 11, 73–84.
- Oldroyd B.P. (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees, Trends. Ecol. Evol. 14, 312–315.
- Park Y.L., Kondo V., White J., West T., McConnell B., McCutcheon T. (2009) Nest-to-nest dispersal of *Chaetodactylus krombeini* (Acari, Chaetodactylidae) associated with *Osmia cornifrons* (Hym., Megachilidae), J. Appl. Entomol. 133, 174–180.
- Qu D., Maeta Y., Goubara M., Nakatsuka K.J., Kozo J., Kenji K. (2002) Reproductive strategy in

- the two species of cleptoparasitic astigmatid mites, *Chaetodactylus nipponicus* and *Tortonia* sp. (Acari: Chaetodactylidae and Suidasiidae), infesting *Osmia cornifrons* (Hymenoptera: Megachilidae). I. Invasion/infestation patterns and partial use of the host food, Jpn J. Entomol. 5, 121–141.
- Roubik D.W. (1987) Notes on the biology of anthophorid bee *Tetrapedia* and the mite *Chaetodactylus panamensis* Baker, Roubik and Delfinado- Baker (Acari: Chaetodactylidae), Int. J. Acarol. 13, 75–76.
- Schwarz H.H., Huck K. (1997) Phoretic mites use flowers to transfer between foraging bumblebees, Insectes Soc. 44, 303–310.
- Trouessart E.L. (1904) Sur la coexistence de deux formes d'Hypopes dans une même espèce, chez la Acariens du genre *Trichotarsus*,. C. R. Soc. Biol. 56, 234–237.
- Vicidomini S. (1996) Biologia di *Xylocopa* (*Xylocopa*) *violacea* (L., 1758) (Hymenoptera: Apidae): interazione con *Sennertia* (*Sennertia*) *cerambycina* (Acari Chaetodactylidae), Boll. Zool. Agr. Bachic. 28, 71–76.