Original article

Thermoregulation in the large carpenter bee *Xylocopa frontalis* in the face of climate change in the Neotropics

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Abstract – There is evidence that climate change may worsen the ecological conditions needed by many pollinator species for their survival; however, little is known about how the rise in ambient temperature might affect the survival of large bee species in tropical regions of the planet. This study investigated the thermoregulation mechanisms of the large carpenter bee *Xylocopa frontalis* when nesting spontaneously in nests designed to allow direct measurement of the bees' temperature while carrying out different activities. It was seen that the species divides its activities throughout the day according to the amount of body heat generated and the ambient temperature, performing activities that generate more body heat at cooler times of the day. The bees also use strategies of thermoregulation, such as heat loss by air convection, evaporative cooling by nectar dehydration, and not foraging during the hottest times of the day to avoid overheating. The study shows that *X. frontalis* is well adjusted to the ecological conditions of the areas in which it lives but suggests that future rises in the ambient temperature could pose a serious threat to the survival and pollination services provided by this bee and other similar tropical species.

1. INTRODUCTION

Pollinators are essential for the reproduction of wild plants and for food production around the world, with approximately 35% of agricultural production and 87.5% of flowering plants depending to some degree on these agents (Klein et al. 2007, Klein et al. 2020; Ollerton et al. 2011; Potts et al. 2016). However, pollinators are in decline all over the world due to a variety of reasons, such as habitat loss and fragmentation, pests, diseases, invasive species, pesticides and climate change (Freitas et al. 2009; Meeus et al. 2012; Potts et al. 2016; Brown et al. 2016).

Among these, climate change is worrisome, as it can compromise pollination services on a global scale via isolated extreme events, such as fires, storms and hurricanes, but mainly due to global warming and a gradual rise in the mean temperature in different regions of the planet. Such events can compromise the survival of pollinators, leading to significant reductions in population (Freitas et al. 2009; Rasmont and Iserbyt 2012; Oliver et al. 2015; Brown et al. 2016), or to temporal mismatch between pollinators and the flowering of crops, which

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represents a great risk to agricultural production and food security (Settele et al. 2016; Bezerra et al. 2019a).

Bees are considered the principal pollinators, and there is evidence that climate change, especially global warming, may worsen the ideal ecological conditions of many species, leading to local extinctions and a reduction in areas of natural occurrence (Herrera et al. 2014; Polce et al. 2014; Giannini et al. 2013, 2017; Elias et al. 2017; Bezerra et al. 2019a; Soroye et al. 2020). According to the International Panel on Climate Change (IPCC) (2013), an increase of between 0.3 and 4.8 °C in mean global temperature may occur by 2080. Although social bees may have difficulties in dealing with this phenomenon, populations of solitary bees are at greater risk of being affected, as they depend more on the ambient temperature to regulate body heat and go through their hibernation/aestivation cycles (Stone 1993, 1994; Heinrich and Esch 1994; Bartomeus et al. 2011). However, investigation into the potential impact of climate change on bees is usually focused on small solitary species native to temperate regions of the planet (Stone 1993; Bishop and Armbruster 1999; Bartomeus et al. 2011; Soroye et al. 2020).

One of the few studies carried out with large bees in a tropical environment was conducted by Bezerra et al. (2019a) and showed that increases between 0.3 and 4.8 °C in mean temperature due to climate change, as predicted by the IPCC (2013), may reduce by 47.9 and 35.3% the areas suitable for survival of the carpenter bee *Xylocopa frontalis* (Olivier 1789) and *X. grisescens* (Lepeletier 1841) respectively by 2080. Large species of genus *Xylocopa* are essential pollinators of wild and cultivated plants with large flowers, such as crotalaria (*Crotalaria juncea* L.), passion fruit (*Passiflora edulis* L.), Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.), annatto (*Bixa orellana* L.) and pitaya (*Hylocereus* spp.), as their size enables the reproductive structures of these flowers to be reached while the bees perform or improve pollination, with consequent results on the quantity and quality of the produced fruit (Freitas et al. 2017; Cavalcante et al. 2018; Muniz et al. 2019; Klein et al. 2020). These bees are also important pollinators for those plant species that rely on buzz pollination, such as crotalaria and annatto.

As *X. frontalis* and *X. grisescens* are large species, and the females are predominantly black despite living in tropical regions, difficulties in thermoregulating their body temperature due to predicted increases in the mean ambient temperature may be related to the anticipated reduction in areas suitable for these species, as suggested by Bezerra et al. (2019a). In fact, Chappell (1982) found that *X. californica* changes its foraging pattern once the ambient temperature exceeds 41 °C, a value that is normal at the hottest times of the day in some areas of the Brazilian countryside (Giannini et al. 2017; INMET 2020). However, there are no records of *X. frontalis* being found where the maximum ambient temperature regularly reaches 35 °C or higher.

Thermoregulation in *Xylocopa* has been little studied, and there is a lack of information under tropical conditions or situations of free nesting. These bees nest by excavating galleries inside dead wood (Gimenes et al. 2006; Marchi and Melo 2010; Junqueira et al. 2013; Silva et al. 2014), thereby preventing access for their body temperature to be measured inside the nest. As such, the few existing studies were conducted in laboratories or even in the field, with the bees kept in confined environments or attached to equipment for measuring body temperature and body heat (Baird 1986; Heinrich and Buchmann 1986; Volynchik et al. 2006). Restricting individuals invariably causes stress, contributing to a rise in temperature, and potentially leading to overestimated results (Stabentheiner et al. 2007).

However, recent studies have proposed models of nesting devices (trap nests) for breeding *Xylocopa* bees that allow adult individuals inside the nests to be observed and accessed (Oliveira-Filho and Freitas 2003; Freitas and Oliveira-Filho 2003; Pereira and Garófalo 2010; Junqueira et al. 2013; Maués et al. 2015; Silva and Freitas 2018). One such model consists of a simple wooden bar where the bees build their nests and might reuse for up to three generations (Bezerra 2018). With this model, it is possible to observe the bee performing its daily activities inside the nest, allowing the temperature of the bee to be

measured using a thermal imager and laser thermometer.

The present study attempted to investigate thermoregulation in *Xylocopa frontalis* nesting freely in nests that allow the direct measurement of their body temperature when performing the various activities necessary for their survival and reproduction in a given environment. The aim was to investigate the thermoregulation processes of these bees in a hot environment without the stress caused when they are restrained and attempt to understand how these processes are related to the predicted reductions in areas where *X. frontalis* occurs, due to a rise in the mean ambient temperature in the near future. This study can help in understanding how climate change may affect large bees in the tropical regions of the planet.

2. MATERIAL AND METHODS

2.1. Study area

The study was carried out during August, September, October and December 2018, and January and February 2019, at the Bee Unit of the Federal University of Ceará, in the city of Fortaleza, Brazil (03° 43′ 02″ S and 38° 32′ 35″ W, at an altitude of 16 m) (IPECE 2019).

Fortaleza is located in the coastal region of the state of Ceará. The climate is hot tropical sub-humid, with a rainy period from January to May, a mean annual rainfall of 1338.0 mm, a mean temperature of 26.9 °C and a mean annual minimum and maximum of 23.6 and 30.4 °C respectively; the relative humidity is 78%, with 2856.5 h of sunshine per year and a wind speed of 3.7 m/s (IPECE 2019).

The carpenter bees used in this experiment belong to species *Xylocopa frontalis*, common in the Neotropics, and found in areas with maximum temperatures that reach 35 °C or less (Figure 1). They were housed in nests built of wooden bars placed in a bee breeding facility (Xylocopary), and in a similar, but smaller structure, in a small forest fragment, both located within the area of the Bee Unit. The Xylocopary consists of a 5-m × 15-m structure, build in a wooden shed and covered with tiles of fibre cement. The forest is a remnant of native vegetation,

characterised as a vegetation complex of the Coastal Zone, of approximately 7000 m². The breeding facility located there is similar to the Xylocopary, but much smaller, measuring 1 m × 1.65 m.

2.2. *Xylocopa* nests

The nests used in the experiment were based on the model used by Bezerra (2018) and consist of 30 cm × 5 cm × 5 cm bars of pinewood, giving a total volume in the wood of 750 cm³. *Pinus* was chosen for the experiment, as it is a relatively soft substrate, facilitating excavation by the carpenter bees. Each wooden bar had a perforation, 2 cm in diameter, with depths that varied from 3 to 11 cm to stimulate nesting. The bars were stacked and grouped to form blocks consisting of several nests (Figure 2). During the experiment, a minimum of 23 and a maximum of 36 nests were inhabited at any one time, and measurements were taken randomly as the bees were performing some of the activities under investigation, during the days and times of observation.

2.3. Thermographic analysis

Being linear, the nest used made it possible to take infrared images of the bees carrying out their activities in and around the nest throughout the day, in order to carry out the thermographic analysis. For this purpose, a HOT TEC® HT31 Thermal Imager was used with an FPA (Focal Plane Array) uncooled microbolometric detector with a resolution of 19,200 pixels, accuracy of 2% and thermal sensitivity of 0.06 °C, which was aimed directly at the bee inside or near the nest to record the body temperature.

Ranges of body temperature were measured for the different types of bee behaviour, i.e. at rest inside the nest, returning from the field after foraging, excavating the wood to build the nest/cells, dehydrating nectar, and during the process of warming up the flight musculature (pre-flight warm-up) before leaving for the field. The value for emissivity used to calibrate the thermal imager in the experiment was 0.97, the same as previously determined in other bee species (Stabentheiner and Schmaranzer 1987).
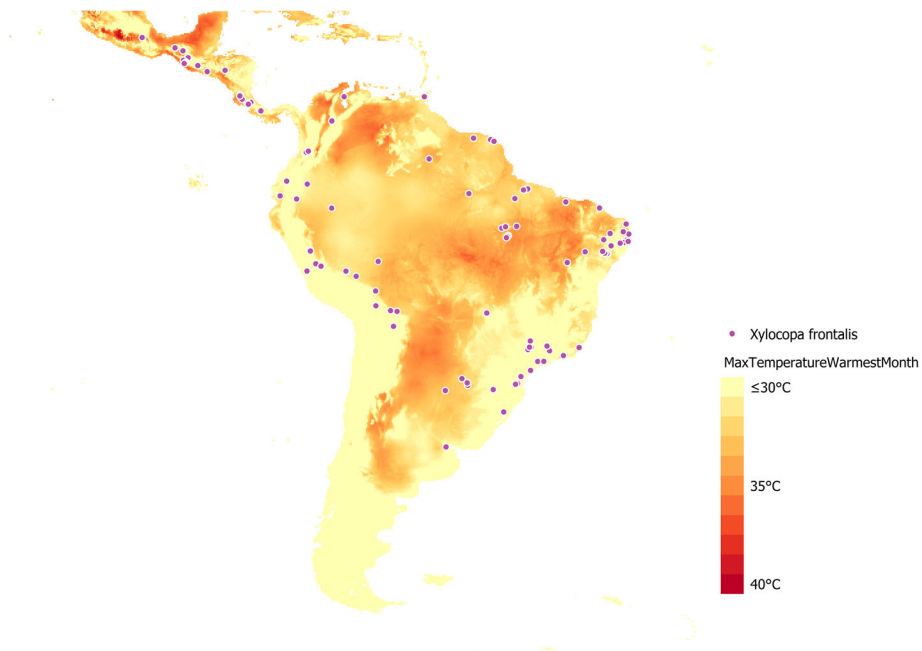
Figure 1. Thermal map of maximum ambient temperatures in the Neotropics and records of the occurrence of *Xylocopa frontalis*. No occurrence is seen in areas where the maximum ambient temperature reaches 35 °C or higher. Sources: Fick and Hijmans (2017), Bezerra et al. (2019b).

The measurements were made in periods of 3 h (04:30 to 07:30, 07:30 to 10:30, 10:30 to 13:30, 13:30 16:30 and 16:30 to 19:30). The bees were observed for only one of these periods each day, drawn the previous evening, taking care to avoid repeating any time period in any one week, so that measurements for that week were taken at a different time each day. The observations were made from Monday to Friday, giving a total of 15 h observation per week. These time intervals include the entire period of activities performed by the bees during the day under the conditions of the study. Measurements were taken with the observer positioned in front of the nests so as to see all of them and take thermal pictures every time a bee presented any of the above behaviour. As each nest has only one adult bee for most of the time, it was possible to avoid pseudoreplication, recording each bee only once for a given behaviour per period of observation. The data were
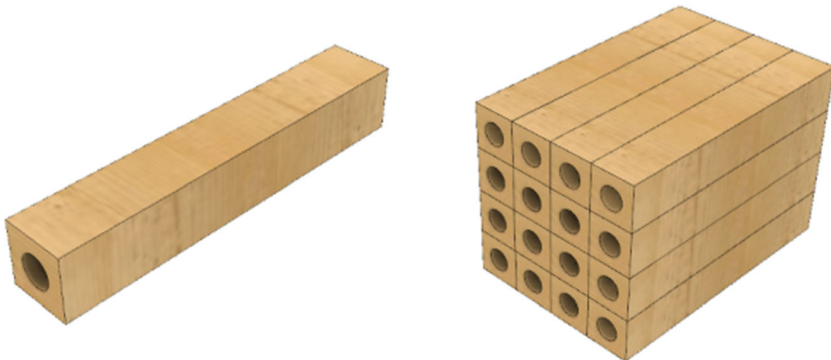

Figure 2. Schematic representation of the nests used for *Xylocopa frontalis*.

collected during the first and third weeks of each month, for 6 months, three during the dry season and three during the rainy season, for a total sampling period of 180 h. This period includes the peak of the dry season (August, September and October), characterised by an abundance of resources in the field, and the start of the rainy season (December, January and February) when the rainfall regime does not yet interfere in the bees' period of activity. Each measurement was recorded as a thermal picture, and a total of 3626 pictures were taken. Of these, 784 images (imprecise bee identity, bee behaviour, possible pseudoreplications and images not showing the bee thorax) were discarded, leaving 2842 images to be processed.

The IR Reporter® software was used to analyse the images generated by the thermal imager. Circular or rectangular shapes were created around the thorax, the head or the abdomen of the bee, approximating their shape as much as possible, but ensuring that only bee pixels were selected (Volynchik et al. 2006). From these figures, it was possible to determine the minimum, maximum and mean temperature of the bee. On many occasions, images of each part of the bee's body (head, thorax and abdomen) could not be taken, as the bees were entirely or partially inside, or entering, the nests, and only one or two body parts could be seen. As a result, a mean body temperature was used in the study, calculated by considering only images where at least the thorax and head or thorax and abdomen were visible, as the thorax generates heat and is generally hotter than the head or the abdomen. In addition, weather data for the period (mean ambient temperature: 27.56 °C; maximum ambient temperature: 33.4 °C; relative humidity: 68.5%; solar radiation: 4333.76 kJ m^{-2}; rainfall: 77.15 mm; wind speed 2.77 m s^{-1}) were obtained from the Weather Station of the Federal University of Ceará, located 500 m from the study site.

2.4. Statistical analysis

After processing the images, the data were tabulated. Variations in the mean, minimum and maximum body temperature, and the ambient temperature were analysed between the different types of bee behaviour. The variations in temperature were also verified between the months of study, the time of day and the location of the nests. The Kruskal and Wallis (1952) was applied to check whether the temperatures differed significantly (at a level of 5%) between behaviour types, the months of study, the time of day or nest location. This test was used due to the non-normality of the data and the presence of outliers. When the Kruskal-Wallis test was significant for variables of more than two categories, Dunn's post hoc test (Dunn 1964) was applied to check for significant differences. The same statistical tests were used to analyse how the temperatures varied between behaviour types over the months of the study, time of day and nest location.

3. RESULTS

Among the types of behaviour observed in this study for which the body temperatures of the bees were taken, the most frequent was resting inside the nest, comprising the majority of the observed events, followed by returning from the field after foraging, pre-flight warm-up, nectar dehydration and others (Table I; Figure 3).

This behaviour occurred at all times of the day; however, different types of behaviour were concentrated at certain times (Table I, Figure 4). During the cooler hours of the day, the bees gave preference to the activities of returning from the field after foraging, excavating the nest and pre-flight warm-up. At the hottest times, it was possible to find a greater number of bees at rest or working on nectar dehydration (Figure 4).

Despite finding that the carpenter bees preferred foraging activities during periods of the day with milder temperatures, especially in the early morning, it was also possible to see such activities being carried out during the hottest hours, when the temperature was greater than 37 °C (Table II), although far less frequently.

The pre-flight warm-up was most often seen before and during the first hours after sunrise, generally when the ambient temperature was still below 30 °C (Table II). At all times, the bees reached a mean body temperature greater than 30 °C before beginning their flight (Table III). On the other hand, nest excavation, although also

Table I. Number of events and percentage value for body temperature recorded for some types of behaviour in *Xylocopa frontalis* throughout the day, in Fortaleza, Ceará, Brazil, from August 2018 to February 2019

Time	Behaviour					
	DN (%)	*E* (%)	*PA* (%)	*R* (%)	*VC* (%)	*O* (%)
04:30–07:30	5 (10.0)	3 (4.7)	52 (69.3)	386 (17.0)	124 (33.3)	0 (0.0)
07:30–10:30	6 (12.0)	7 (11.0)	11(14.7)	462 (20.3)	110 (29.5)	2 (20.0)
10:30–13:30	17 (34.0)	3 (4.7)	5 (6.7)	512 (22.6)	58 (15.5)	2 (20.0)
13:30–16:30	17 (34.0)	17 (26.5)	4 (5.3)	570 (25.1)	62 (16.6)	2 (20.0)
16:30–19:30	5 (10.0)	34 (53.1)	3 (4.0)	340 (15.0)	19 (5.1)	4 (40.0)
Total	50 (100.0)	64 (100.0)	75 (100.0)	2.270 (100.0)	373 (100.0)	10 (100.0)

Values in parentheses refer to the percentage of behaviour performed for time of day within each behaviour. *DN*, dehydrating nectar; *E*, excavating nest; *PA*, pre-flight warm-up; *R*, resting inside the nest; *VC*, returning to the nest from a foraging trip; *O*, others

carried out under mild temperatures, was concentrated in the late afternoon, when the ambient temperature started to decline. When excavating the nest, the bees showed a mean body temperature of around 29–30 °C (Table III). Resting in the nest and nectar dehydration were concentrated at the hottest times of the day, with the mean ambient temperature around 30 °C, while the temperature of the bee reached close to 35 °C (Tables II and III).

With regard to the mean body temperature of the bees, a pattern could be seen in temperature between the behaviour types, despite variations for each activity (Figures 4, 5 and 6; Table III). Bees at rest and digging the nest showed a similarly lower mean body temperature (27 to 31 °C) compared to bees in pre-flight warm-up or returning to the nest after foraging (28 to 35 °C) (Table III). For nectar dehydration, there was a wide variation in the body temperature of the carpenter bees (28 to 38 °C) (Table III).

Returning to the nest after foraging was the activity with the highest mean body temperature in *X. frontalis*, while the lowest mean temperature was seen in the bees at rest inside the nest (Table III). The mean body temperature of bees returning to the nest after foraging differed significantly ($p < 0.05$) from the temperatures observed for the other activities, both in relation to the time of day, the month of observation and nest location (Figures 4, 5 and 6; Table III). The other types of behaviour showed similar mean body

temperatures, with significant differences only in specific cases, as can be seen in Table II. Among the types of behaviour under evaluation, resting in the nest showed the highest correlation between mean body temperature and ambient temperature (0.5483207, $p < 0.001$) (Figure 7).

There was no significant difference in ambient temperature as a function of nest location ($p < 0.05$), in the same way that no statistical difference ($p < 0.05$) was seen for activities carried out by the bees, comparing the mean body temperature between bees housed in the forest and those in the Xylocopary breeding facility, with the exception of resting in the nest (Figure 5; Table III). (Additional data are available in Online Resource 1.)

With regard to the time of year, the highest mean body temperatures were recorded during October and December, when the bees returned from the field (greater than 34 °C), and February, when dehydrating nectar (greater than 38 °C) (Table IV). However, in terms of higher absolute body temperature, values above 40 °C were recorded each month for the behaviour of returning from foraging, reaching almost 47 °C for one event in September (Figure 6; Table IV). With the delay in rainfall, September, October and December corresponded to the peak of the dry season that year, and achieved the highest ambient temperatures (Table IV).

In addition to the more frequent types of behaviour, it was possible to see sporadic stressing behaviour, such as bees fighting at
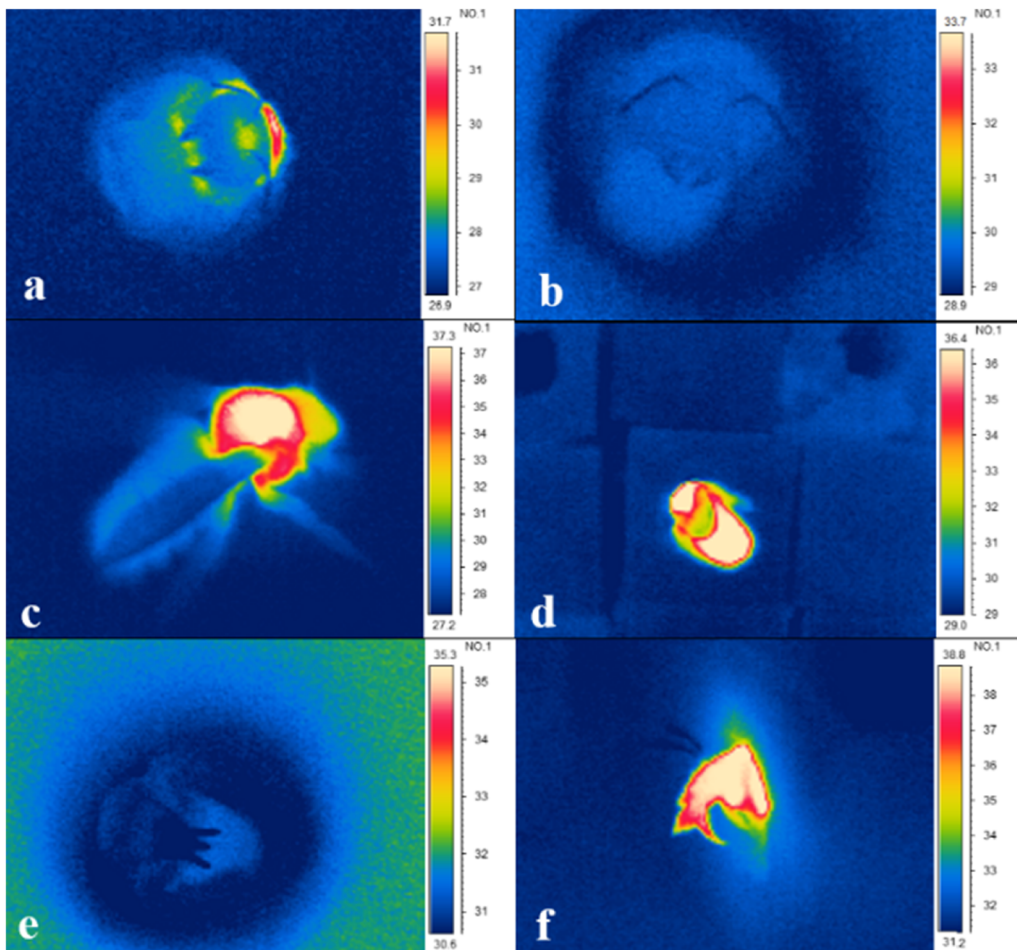
Figure 3. Thermographic images for frequent behaviour presented by *Xylocopa frontalis*: nest excavation (**a**), resting inside the nest (**b**), pre-flight warming (**c**), returning to the nest after foraging (**d**), dehydrating nectar received through trophallaxis (**e**) and dehydrating nectar immediately after returning from the field (**f**).

the entrance to the nest, responding to attacks by ants, warming up in the sun after a possible eviction from the nest, and audibly vibrating inside the excavated gallery (Figure 8). In these situations, there was a rapid increase in individual body temperature. In cases where the nests were attacked by ants or the bees were seen fighting another bee at the entrance to the nest, a fast rise in their thorax temperature to 5.35 °C and 4.65 °C above the ambient temperature could be seen respectively. One bee found on the ground was a young female, probably evicted from the nest by its mother, which remained in the sun for a few minutes and then took

flight once the temperature of the thorax had increased from 30.8 °C, to 36.1 °C. Finally, a bee that suddenly started intense audible vibration inside the nest for no obvious reason, showed a 10.4 °C increase in thorax temperature (Figure 8).

4. DISCUSSION/CONCLUSION

The great majority of observations (approximately 80%) showed the bees at rest inside the nest. This may be due to the fact that resting is a long-lasting activity in relation to the other activities, increasing the chances of this behaviour being seen where there are several *Xylocopa*
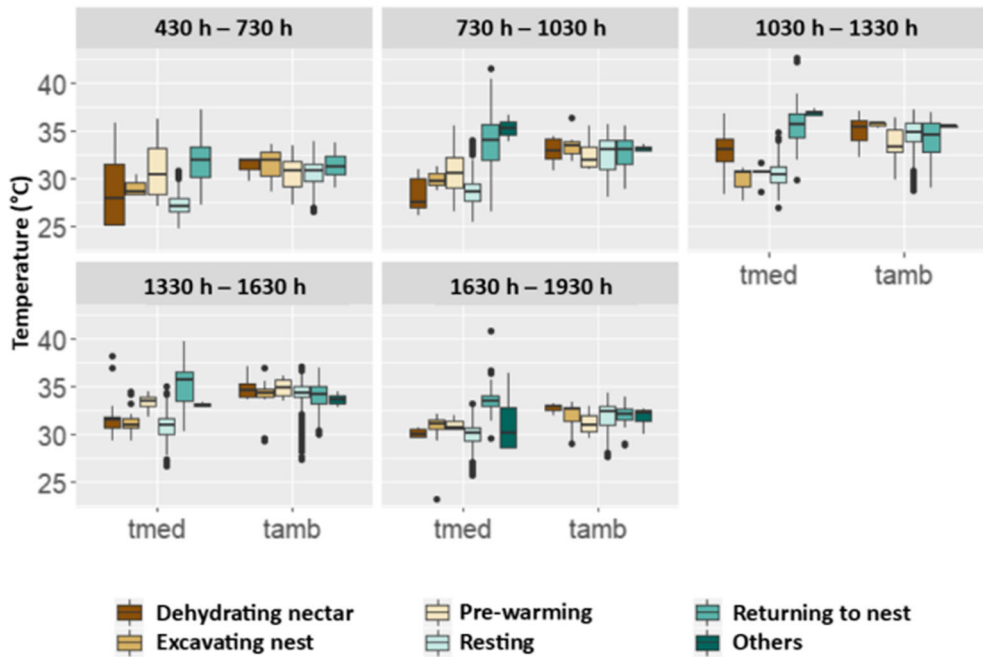
Figure 4. Mean ambient temperature and mean body temperature in *Xylocopa frontalis* when performing different types of behaviour in the nests at different times of the day.

Table II. Mean, maximum and minimum ambient temperatures for time of day, month of observation and nest location, in Fortaleza, Ceará, Brazil, from August 2018 to February 2019

		Ambient temperature		
		Maximum	Minimum	Mean ± standard deviation
Period	04:30–07:30	33.9	26.9	31.5 ± 1.5[a]
	07:30–10:30	36.4	28.1	32.8 ± 1.9[c]
	10:30–13:30	37.2	28.8	33.0 ± 1.9[e]
	13:30–16:30	37.1	27.3	32.9 ± 2.1[d]
	16:30–19:30	34.3	27.6	31.8 ± 1.5[b]
Month	August	37.1	28.6	33.1 ± 2.0[b]
	September	37.2	26.6	33.7 ± 2.3[c]
	October	35.9	27.3	33.3 ± 1.9[bc]
	December	36.8	29.2	33.2 ± 1.8[b]
	January	35.6	27.3	32.4 ± 2.2[a]
	February	35.5	27.9	32.6 ± 1.9[a]
Place	Forest	37.2	26.6	32.9 ± 2.1[a]
	Xylocopary	36.9	28	32.8 ± 2.1[a]

Mean values followed by the same lowercase letter in the column do not differ by Kruskal-Wallis test at 5%

Table III. Mean body temperature of *Xylocopa frontalis* when performing some types of behaviour in the nests in a coastal area of Ceará, for time of day, month of observation and nest location

		Behaviour				
		DN	E	PA	R	VC
Period	04:30–07:30	29.14 ± 3.04^{abAB}	29.1 ± 1.22^{aaAB}	30.84 ± 2.85^{aB}	27.25 ± 1.97^{aaA}	31.93 ± 2.24^{aC}
	07:30–10:30	28.31 ± 0.99^{aA}	29.94 ± 0.96^{aaAB}	30.83 ± 2.62^{aB}	28.67 ± 1.44^{bA}	33.98 ± 2.65^{bC}
	10:30–13:30	32.82 ± 2.12^{cAB}	29.83 ± 1.85^{aA}	30.52 ± 1.10^{aA}	30.49 ± 1.27^{dA}	35.64 ± 2.19^{bB}
	13:30–16:30	32.17 ± 2.82^{bcA}	31.32 ± 1.43^{aA}	33.27 ± 1.15^{aAB}	30.79 ± 1.31^{eA}	35.19 ± 2.09^{bB}
	16:30–19:30	30.10 ± 0.48^{abcAB}	30.75 ± 1.50^{aB}	31.00 ± 0.79^{aABC}	29.90 ± 1.40^{cA}	33.93 ± 2.38^{abC}
Month	August	29.95 ± 3.48^{aAB}	31.03 ± 0.65^{aB}	30.87 ± 2.63^{abB}	29.31 ± 2.01^{aA}	32.74 ± 3.13^{aB}
	September	30.98 ± 1.97^{aB}	31.07 ± 1.04^{aB}	30.90 ± 2.85^{abAB}	29.55 ± 1.68^{abA}	33.82 ± 3.01^{abC}
	October	31.05 ± 2.63^{aA}	29.72 ± 3.20^{aA}	32.86 ± 2.53^{bcAB}	30.28 ± 1.74^{cA}	34.78 ± 2.65^{bB}
	December	32.14 ± 2.93^{aAB}	31.32 ± 0.23^{aAB}	34.10 ± 0.00^{abAB}	29.81 ± 1.75^{bA}	34.04 ± 2.15^{bB}
	January	34.35 ± 3.60^{aB}	-	28.88 ± 0.98^{aA}	29.14 ± 1.77^{aA}	33.56 ± 2.73^{abB}
	February	38.15 ± 0.07^{aBC}	29.90 ± 1.11^{aAB}	30.81 ± 2.27^{abABC}	29.22 ± 1.65^{aA}	33.82 ± 2.29^{bC}
Place	Forest	30.75 ± 2.61^{aB}	30.48 ± 1.50^{aB}	30.98 ± 2.69^{aB}	29.18 ± 1.74^{aA}	33.68 ± 2.84^{aC}
	Xylocopary	32.52 ± 2.89^{aBC}	31.27 ± 1.13^{aABC}	30.82 ± 2.53^{aAB}	30.16 ± 1.80^{bA}	33.84 ± 2.60^{aC}

Mean values followed by the same lowercase letter in a column and uppercase letter on a row differ by Kruskal-Wallis test at 5%. *DN*, dehydrating nectar; *E*, excavating nest; *PA*, pre-flight warm-up; *R*, resting inside the nest; *VC*, returning to the nest from a foraging trip
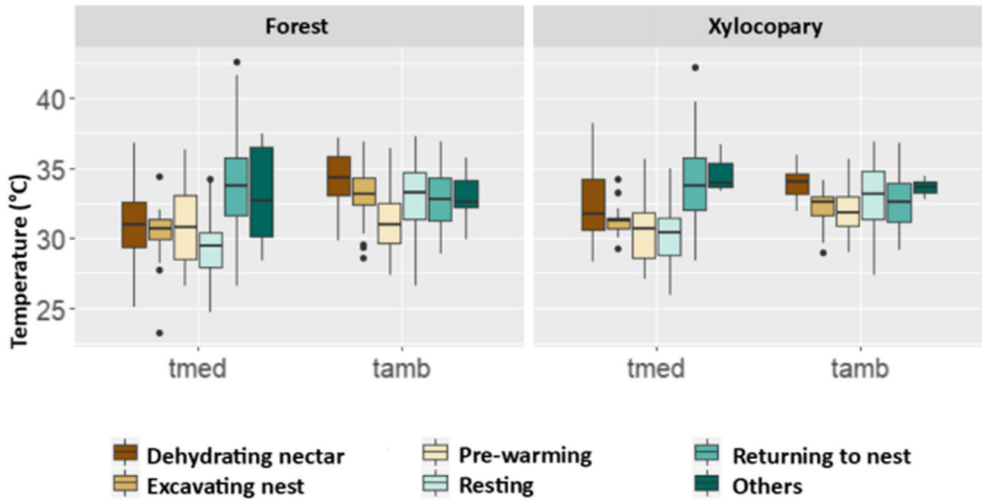
Figure 5. Mean ambient temperature and mean body temperature of *Xylocopa frontalis* when performing different types of behaviour in the nests at different locations.

nests, as in this study (Camillo and Garofalo 1982; Freitas et al. 2017); or these bees, being solitary, producing few offspring and not accumulating considerable amounts of food resources (honey and pollen) in the nest, do not need to be so active in search of food (Oliveira-Filho and Freitas 2003; Silva et al. 2014); or even because it is an adaptive strategy to minimise activities that generate heat, especially at the hottest times of the day, as seen in other species from environments with high
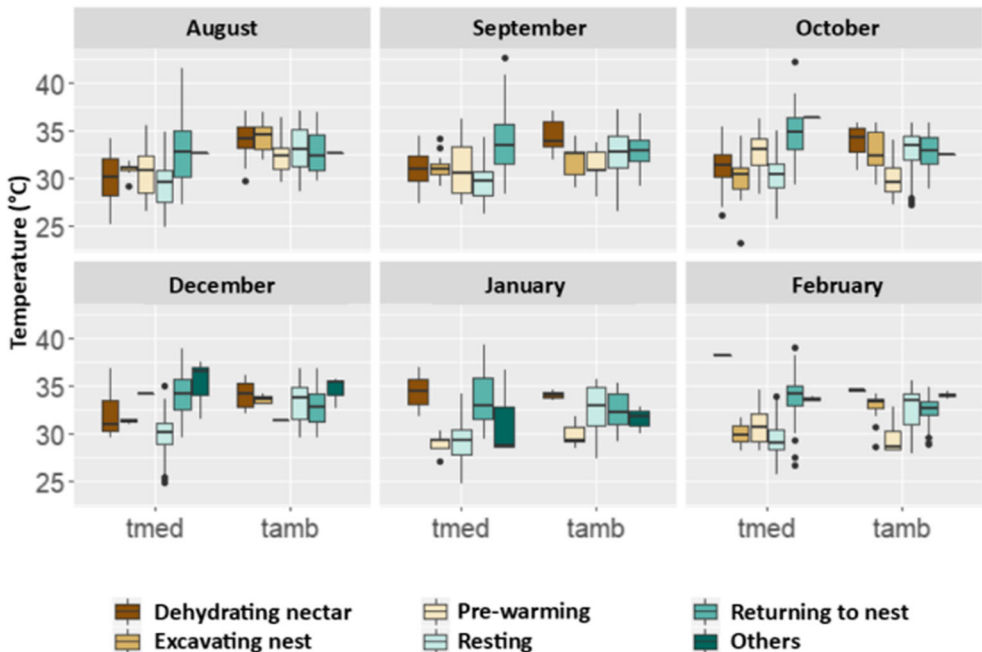

Figure 6. Mean ambient temperature and mean body temperature of *Xylocopa frontalis* when performing different types of behaviour in the nests during different months of the year.
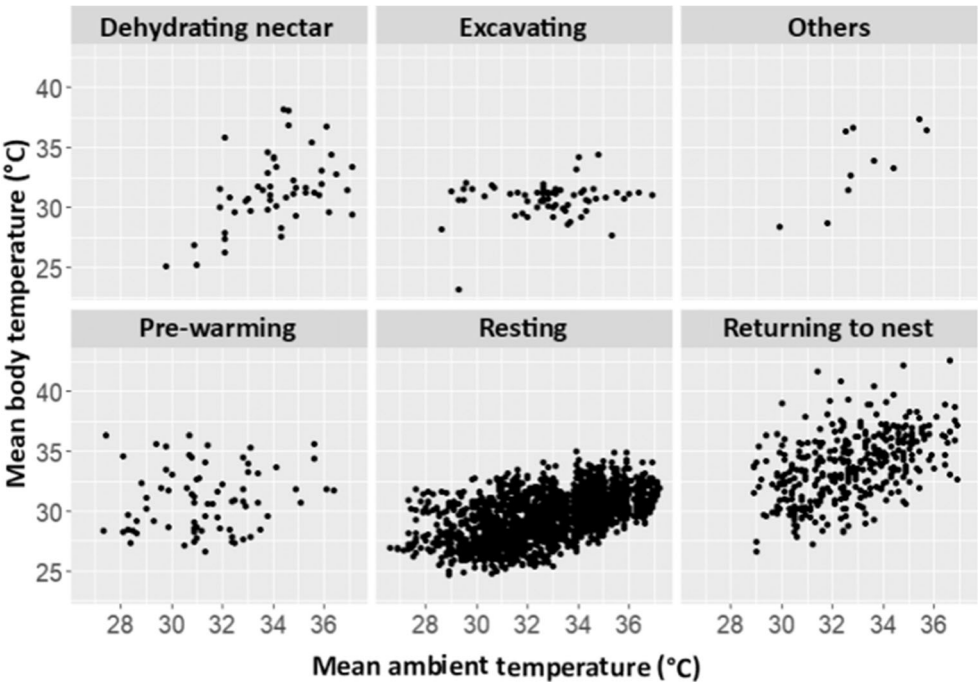
Figure 7. Correlation between the mean ambient temperature and mean body temperature of *Xylocopa frontalis* when performing different types of behaviour in the nests located in Fortaleza, Ceará, Brazil.

Table IV. Maximum and minimum body temperature for the different types of behaviour observed in *Xylocopa frontalis* housed in the nests in the coastal region of Ceará, for the month of observation and time of day

		Behaviour									
		DN		E		PA		R		VC	
		Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
Month	August	35.2	24.5	33.0	28.4	42.2	25.9	35.5	23.9	44.8	26.0
	September	35.5	32.7	34.9	28.5	41.8	26.1	35.2	26.0	46.9	26.9
	October	36.2	25.9	35.2	23.0	44.8	27.6	35.4	25.5	44.3	28.6
	December	38.4	28.9	34.1	29.3	40.9	26.9	35.7	24.3	42.9	26.8
	January	40.2	30.5	-	-	36.0	25.6	35.1	24.5	42.1	36.5
	February	39.9	35.2	32.6	27.6	42.0	27.2	35.7	32.8	41.8	34.3
Period	04:30–07:30	38.4	24.5	31.1	27.7	44.8	25.6	31.9	24.2	42.0	23.4
	07:30–10:30	32.5	25.9	33.0	28.1	37.7	26.1	35.1	25.2	46.9	25.2
	10:30–13:30	37.9	27.7	31.4	30.7	34.0	28.0	35.7	26.4	45.6	28.9
	13:30–16:30	40.2	28.7	35.2	27.6	38.0	29.6	35.7	26.2	42.9	28.5
	16:30–19:30	31.0	28.9	34.1	23.0	32.2	29.9	34.8	24.9	43.3	28.7

DN, dehydrating nectar; *E*, excavating nest; *PA*, pre-flight warm-up; *R*, resting inside the nest; *VC*, returning to the nest from a foraging trip
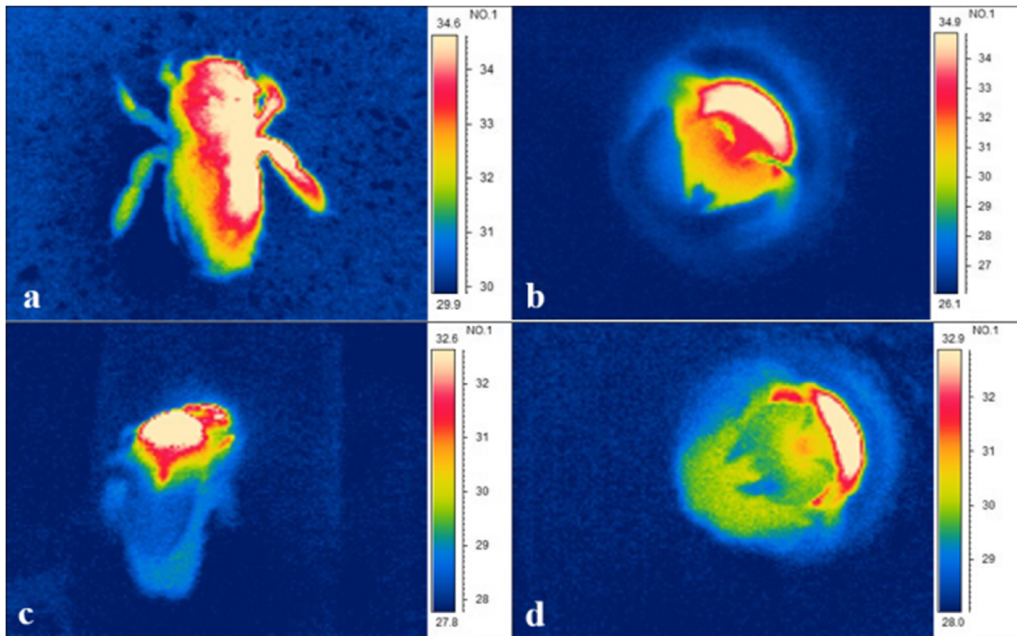
Figure 8. Thermographic images for occasional behaviour performed by *Xylocopa frontalis* : basking in the sun (**a**), under ant attack (**b**), fighting other bee at the entrance to the nest (**c**) and vibrating inside the nest (**d**).

temperatures (Heinrich and Buchmann 1986; Heinrich 1993).

In fact, activities that generate more body heat (foraging and pre-flight warm-up) were concentrated in the cooler hours of the day, while those that produce less or no body heat (rest and excavation of the nest) were more common at higher temperatures, corroborating the findings of Chappell (1982), and Heinrich and Buchmann (1985) for other species of *Xylocopa* . However, all behaviour types were seen throughout the day, suggesting that this species of carpenter bee may have preferential times for different activities, but reproductive needs and probably the availability of food in the field seem to be factors that cause them to carry out certain activities at times of the day which are less favourable to regulating body temperature.

With regard to thermoregulation, for most of the time, the bees kept their body temperature above the ambient temperature even when performing activities that generated little heat. The exception was when the bees were resting in the nest, which showed some correlation between body temperature and the ambient temperature. But this can be explained by the animals

displaying temporal endothermy, being considered ectothermic when not performing any activity; when at rest, their body temperature remains close to the ambient temperature (Heinrich 1993; Heinrich and Buchmann 1986).

However, pre-flight warm-up is necessary in order to fly because the flight musculature of these bees does not function properly at temperatures below 30 °C (Heinrich 1993). In fact, the mean pre-flight warm-up temperatures seen were always slightly above this figure, although maximum values above 40 °C were recorded throughout the study period, except during January. Nevertheless, these higher values were observed during the early hours of the day, when the ambient temperature is still low. In large bees, internal heat generation is more efficient than in small bees. In contrast, loss of heat by convection is greater in bees with less body mass. As such, the efficient endothermy of *Xylocopa* , especially in the early hours of the morning, combined with the large body mass of these bees, allows them to forage under conditions which would be impossible for smaller bees, especially at low temperatures (Gerling et al. 1983; Somanathan and Borges

2001; Silva et al. 2019). For the conditions of this study, when even before sunrise the ambient temperature remained above 23 °C, low temperatures cannot be considered a limiting factor to these bees starting their activities, but show the need for pre-flight warm-up at temperatures that favour the correct functioning of the flight musculature, and minimise heat loss by convection when flying while the ambient temperature is below 30 °C. In this situation, the dark colour of the bee favours greater heat absorption when exposed to the sun, helping it to reach and maintain the minimum body temperature necessary for flight (Figure 8a).

On the other hand, the body temperature of the bees when they returned from field trips showed high mean values, reaching 35 °C, with maximum values greater than 42 °C, reaching close to 47 °C. These figures probably explain the preference showed by the carpenter bee to forage during the coolest hours, avoiding the hottest times of the day. The high body temperature of these large bees when returning from the field is due both to the internal heat generated due to flight, resulting from the metabolism of the animal and by exposure to solar radiation (Chappell 1982; Baird 1986; Heinrich and Buchmann 1986; Corbet and Huang 2016). Despite this, the females of *X. frontalis* are coloured black, which may seem a disadvantage for bees living in regions of tropical climate under high temperatures and intense solar radiation (but see the above discussion on pre-flight warm-up). However, not only should the colour or body weight be considered in heat generation but also the environment where the bee lives and the phylogeny. It is possible that a flight system tolerant of extremely high working temperatures exists in bees living in warm environments that makes the bee capable of low rates of warm-up, even at relatively high ambient temperatures (Stone and Willmer 1989). Chappell (1982) states that the efficient loss of heat by convection during flight in species of *Xylocopa*, especially those that show little pubescence, would compensate for the heat gained due to the exposure of the black integument to solar radiation. Despite the existence of other heat-loss mechanisms, this seems to be the main and most efficient mechanism for this genus of bee (Chappell 1982; Baird 1986; Heinrich and Buchmann 1986; Volynchik et al. 2006). Referring to this, Heinrich and Buchmann

(1986) demonstrated that *X. varipuncta* flies faster under conditions of high air temperature, increasing its cooling by convection.

Xylocopa is able to transfer heat from the thorax, considered its thermogenic centre, to the head and abdomen, where heat loss occurs through convection (Baird 1986). This heat loss prevents overheating and allows the bee to forage under considerably higher temperatures. This transfer of heat to the head occurs passively, through conduction, but the passage of heat to the abdomen can occur actively, as bees have a mechanism for retaining heat in the thorax consisting of a series of loops in the aorta at the height of the petiole (Baird 1986; Heinrich 1993). Such a mechanism gives considerable control to the transfer of heat from the thorax to the abdomen. In this study, this action was seen in bees that required warming of the flight musculature, retaining heat generated in the thorax with little dissipation to the head (Figure 3c), and in bees returning from foraging trips where the situation was the reverse, the bees needing to dissipate heat from the thorax to the abdomen (Figure 3d).

In addition to the loss of body heat through convection, carpenter bees are also able to lose heat by dehydrating nectar, which, due to the exposure of the collected resource to the air, causes a loss of heat through evaporative cooling (Sakagami and Laroca 1971; Bernardino and Gaglianone 2008). But a wide variation in the body temperature of carpenter bees was seen when they were dehydrating nectar. This happened because, despite *X. frontalis* behaving as a solitary species most of the time, young offspring stay in the nest for some days after emergence, interacting with their mother (parasocial behaviour). Therefore, in this situation, bees resting in the nest, for which the body temperature was close to that of the environment, dehydrated the nectar they received by trophallaxis from other bees (Figure 3e), and bees that returned to the nest after foraging also dehydrated nectar as a way of cooling down their dangerously high temperatures (Figure 3f).

Therefore, the high body temperatures shown by individuals returning from the field irrespective of the time of year or time of day, the concentration of foraging activities at the coldest times of the day, and evaporative cooling by evaporating the nectar to lose body heat, suggest that

X. frontalis is well adjusted to the ecological conditions of tropical regions, but may face difficulties if the ambient temperature rises.

Despite there being no studies that show the limit for the maximum ambient temperature under which *X. frontalis* can thrive, Chappell (1982) found that the foraging pattern of *X. californica* is affected when the ambient temperature exceeds 41 °C. In the present study, conducted in a coastal area, the foraging activity of *X. frontalis* occurred at ambient temperatures ranging between 28.9 and 36.9 °C. As such, the foraging pattern of *X. frontalis* should not be affected by the ambient temperature at any time. However, records from Discover Life, Global Information Biodiversity Facilities (GBIF), Species Link and WorldClim show that *X. frontalis* has not been found in neotropical areas where maximum temperatures reach 35 °C or higher (Figure 1), and the long resting period seen in this study (80% of the observations) may be the result of the bees avoiding activities which might generate heat, especially foraging, during the hottest times of the day.

The IPCC (2013) points to potential increases of between 0.3 and 4.8 °C in mean global temperature by 2080, with the present area of natural occurrence of this species of carpenter bee being one of the most affected. In addition, studies of climate modelling have shown that increases in the mean ambient temperature due to climate change can reduce the areas suitable for the survival of *X. frontalis* by up to 47.9% (Giannini et al. 2013; Bezerra et al. 2019a). Currently, temperatures around or greater than 41 °C have been recorded at the hottest times of the day in some areas of the Brazilian countryside, and even extremely high temperatures of over 50 °C in full sunlight (Maia-Silva et al. 2015; Hrncir et al. 2019; INMET 2020). Moreover, in a study in this region with a small-sized tropical bee, *Melipona subnitida*, Hrncir et al. (2019) demonstrated that the flight muscles may increase the thoracic temperature of foragers by up to 10 °C above the air temperature in the shade. If a similar increase in temperature occurs with *X. frontalis*, the thoracic temperature may reach a dangerously high 45 °C at an ambient temperature of only 35 °C, explaining why the bee is not found where maximum temperatures reach this value or higher.

In addition, the pollination services offered to wild and agricultural plants by *Xylocopa* bees might also be compromised. For example, *X. frontalis* is one of the main pollinators of the yellow passion fruit (*Passiflora edulis*). Its flowers open at noon and close in the evening, requiring the transfer of large amounts of pollen during this short period to obtain large heavy fruit of commercial value (Oliveira-Filho and Freitas 2003). Currently, the carpenter bee risks overheating when visiting flowers at the hottest times of the day, because temperatures that are critical and lethal to insects are affected by the exposure time (Hrncir et al. 2019; Jørgensen et al. 2019). If average temperatures rise further, *Xylocopa* bees may be prevented from flying and pollinating the passion fruit under these environmental conditions, resulting in a plant-pollinator mismatch (Silva and Freitas 2018; Bezerra et al. 2019a). *X. frontalis* adjusting to forage at higher temperatures is unlikely because species which already tolerate high temperatures are more sensitive to increases in the ambient temperature than species having a lower critical temperature (Rezende et al. 2014).

It can therefore be concluded that despite the bees of genus *Xylocopa* showing successful strategies and a refined thermoregulation mechanism that has allowed them to inhabit the desert, semi-arid and tropical regions of the planet, future climate change, especially the rise in ambient temperature, can pose a serious risk to the survival of these bees, particularly *X. frontalis*. Further studies of critical and lethal temperatures, and thermoregulation mechanisms, combined with monitoring populations of this species, are necessary in order to conserve and develop forms of sustainable exploitation of this pollinator.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest   The authors declare that they have no conflict of interest.

La thermorégulation chez la grande abeille charpentière *Xylocopa frontalis* face au changement climatique dans les régions néotropicales

température corporelle / stratégie de butinage / réchauffement climatique / comportement de thermorégulation / tropiques

Thermoregulation bei der großen Holzbiene *Xylocopa frontalis* angesichts der Klimaveränderungen in den Neotropen

Körpertemperatur / Sammelstrategie / globale Erwärmung / Thermoregulationsverhalten / Tropen

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