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Geographic Distribution, Key Challenges, and Prospects for the Conservation of Threatened Stingless Bee *Melipona capixaba* Moure and Camargo (Hymenoptera: Apidae: Meliponini)

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Abstract

The stingless bee *Melipona capixaba* Moure and Camargo is endemic to the Brazilian Atlantic Forest. Its occurrence is restricted to highlands in the Espírito Santo State, and it has possibly the smallest known geographic distribution among the cataloged stingless bees. It is therefore considered to be an endangered species. Perhaps because of its small area of occurrence, or because it was only identified two decades ago, little is known about the biology of this species, its current geographic distribution, or its actual preservation status. Here, we present the results from the largest sampling of *M. capixaba* conducted in its natural habitat. We developed a distribution map by using a geographic information system. Our data indicate that *M. capixaba* is found in the municipalities of Espírito Santo State at altitudes between 800 m and 1,200 m; with annual average temperatures around 18–23°C; precipitation more than 1,200 mm per year; and vegetation cover-type Mountain Dense Ombrophylous Forest, restricted to an area of approximately 3,450 km^2. We observed colonies both in their natural habitat and under conditions of *ex situ* maintenance, and identified the key challenges and prospects for the conservation of this endangered bee.

Introduction

The stingless bee *Melipona capixaba* Moure and Camargo is endemic to the Brazilian Atlantic Forest. The species is popularly known as “uruçu-preta” (black uruçu) because of its dark coloring and as “uruçu-capixaba” in reference to its endemism. It is the only social bee included in the List of Species of Brazilian Fauna Threatened with Extinction (Brasil, 2003) and classified in the category Vulnerable - VU-B1ab (iii): taxon facing high risk of extinction in the wild, geographical distribution whose extent of estimated occurrence is less than 20,000 km^2, severely fragmented, or with known distribution in not more than ten localities. A continuous decline was observed, inferred, or projected in its area of occupation, extension, and/or quality of habitat (Machado et al., 2008). The main threat for this species is massive deforestation in its habitat, which fragments and isolates populations, reduces food sources and nesting areas, and hinders the survival and reproduction of colonies (Silveira et al., 2008).

The factors that threaten *M. capixaba* are local examples of the factors that affect bees globally. There has been a worldwide decline in the population of these pollinators, and this is mainly due to habitat loss and fragmentation, habitat agrochemicals, pathogens, alien species, climate change, and the interactions among these factors (Freitas et al., 2009; Potts et al., 2010). Thus, monitoring the remaining populations of *M. capixaba* might provide information about their long-term population trends and offer interesting perspectives for bee conservation actions on a larger scale.

The currently known geographic range of *M. capixaba* is restricted to the mountainous regions of the state of Espírito Santo in Brazil. Melo (1996) registered the first occurrence of the species and found that it occurred only at higher altitudes (900-1,000 m) and suggested that this is possibly the smallest known distribution among the cataloged stingless bees. Serra et al. (2012) identified native colonies of *M. capixaba*, all found in regions at altitudes between 900 m and 1,200 m. Nogueira et al. (2014) observed the occurrence of *M. capixaba* colonies in regions at altitudes between 900 m and 1,100 m.

The remaining population of *M. capixaba* is drastically reducing, mainly because of loss and fragmentation of its habitat and activities of the local population, which includes removing the hives from the natural environment for beekeeping. A direct consequence of population reduction is the reduction in genetic diversity, which was verified by Nogueira et al. (2014) through assessing microsatellite markers, inter simple sequence repeat (ISSR) markers, and mitochondrial haplotypes. The loss in genetic diversity reduces the evolutionary potential of the species to adapt to environmental changes and endangers its long-term survival.

Considering the risk of extinction of *M. capixaba*, it is essential to gain some insight into the area of its occurrence, conservation status, state of maintenance of colonies, and the risks and threats faced by the bees. Serra et al. (2012) modeled the potential geographic distribution based on a sample of 14 *M. capixaba* wild nests. However, extensive sampling of natural and beekeeper nests inside its natural habitat would provide an accurate idea of the current geographic distribution of the species and of potential areas for its management and conservation status. Here, we present the results of the largest sampling of *M. capixaba* conducted in its natural habitat. Our goal was to confirm the current geographic distribution area of *M. capixaba*, identify potential areas for management and conservation of the remaining populations, and suggest conservation actions for this bee threatened with extinction.

Materials and Methods

Between 2007 and 2014, field surveys were conducted to collect bee specimens. The field surveys had two objectives: (i) identification of wild colonies, i.e., those located in native forests, and (ii) identification of colonies managed by humans that are close to their natural habitats. The points of occurrence were recorded using Global Positioning System (GPS), and spatial analyses were performed using ArcGIS 9.3 ESRI®.

For spatial analyses, free-access Shapefiles were used. The following programs were used: Shapefiles of the digital elevation of the ASTER GDEM program (NASA, 2009) Shapefiles of the physical limits of municipalities and states of the Brazilian federation, IBGE (http://www.ibge.gov.br/home/download/geociencias.shtm); Shapefiles of the Atlantic Forest vegetation and conservation areas MMA - Ministry of Environment (http://mapas.mma.gov.br/i3geo/datadownload.htm); and IBAMA - Brazilian Institute of Environment (http://siscom.ibama.gov.br/shapes/); and Shapefiles of Forest Remnants of the Atlantic Forest provided by SOS Atlantic Forest Foundation and INPE - National Institute for Space Research (http://mapas.sosma.org.br/dados/).

Contour maps and area delineation considering elevation were generated using the *Surface Analysis* method of ArcGIS 9.3 ESRI®. Temperature maps were generated by interpolation of 30-year historical series data (1977–2006) obtained from 110 weather stations located in the Espírito Santo State and neighboring states. The Kriging spherical method of ArcGIS 9.3ESRI® was used to map the variable temperature, considering the range of annual average temperatures in the historical series.

Contour maps, of temperature and vegetation, were compared, and the intersection areas of the three variables were taken into account in the delimitation of the area estimated for the occurrence of *M. capixaba* and in the delimitation of potential areas for reintroduction and management of the species. The extent of the delimited areas were deduced by calculating the irregular polygon areas with the geometric calculation tool of the ARCGis ESRI® 9.3 mapping platform, using the projected coordinate systems SAD´1969 UTM Zone 24S with km^2 as the area unit.

Results and Discussion

We recorded the occurrence of 194 *M. capixaba* colonies, 27 wild nests and 167 beekeeper nests, at 63 sampling points in 12 municipalities of Espírito Santo State, Brazil (Fig 1). In 10 municipalities, the species is found in native forests or is maintained in areas near the site from which they were collected: Afonso Claudio (AF), Alfredo Chaves (AC),

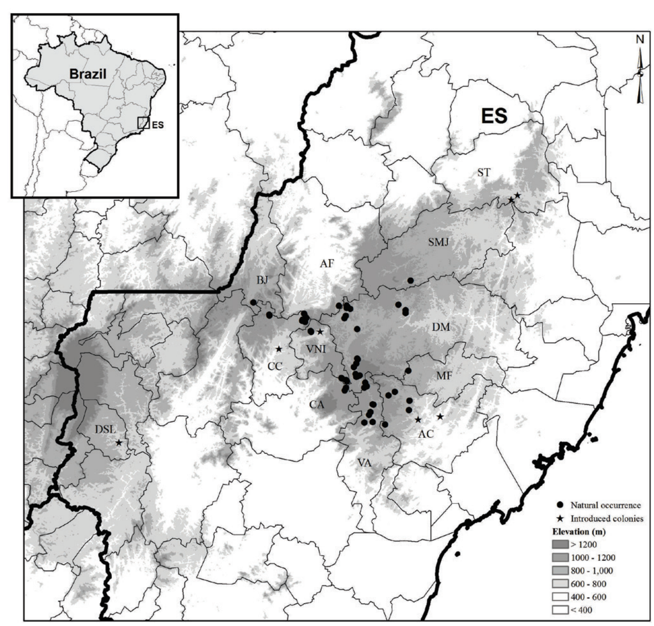

Fig 1. Map of 63 sampling points from 194 colonies of *Melipona capixaba* in the area of current occurrence. Circles on the map represent native or managed colonies close to their natural habitat. Stars on the map represent introduced colonies that were derived from other locations. Legend of municipalities: SMJ - Santa Maria de Jetibá, ST - Santa Teresa, DM - Domingos Martins, MF - Marechal Floriano, AC - Alfredo Chaves, VA - Vargem Alta, CA - Castelo, VNI - Venda Nova do Imigrante, CC - Conceição do Castelo, BJ - Brejetuba, AF - Afonso Cláudio, DSL - Divino de São Lourenço.

Brejetuba (BJ), Castelo (CA), Conceição do Castelo (CC), Domingos Martins (DM), Marechal Floriano (MF), Vargem Alta (VA), Venda Nova do Imigrante (VNI), and Santa Maria de Jetibá (SMJ).

We recorded the occurrence of colonies that were introduced in the municipalities of Divino de São Lourenço (DSL, 4 nests) and Santa Teresa (ST, 8 nests), originated from VNI and SMJ, respectively. Although there are no records of natural colonies in these two locations, these colonies have been successfully maintained for at least 5 years, indicating potential areas of management for conservation near the areas of occurrence.

Geographic distribution

Native colonies of *M. capixaba* were observed at a minimum altitude of 882 m and a maximum altitude of 1,168 m, and no data from naturally occurring colonies or managed colonies were found in regions above 1,200 m and below 600 m (Table 1, Fig 2A).

Although natural occurrence has been observed only between 800 m and 1,200 m, colonies of *M. capixaba* are managed with relative success at altitudes between 700 m and 800 m in colder regions and near native forests. At 3 sites, we verified the maintenance of colonies between 600 m and 700 m (minimum altitude, 612 m), but in these cases, management requires special care with food supplementation. To the best of our knowledge, *M. capixaba* colonies do not remain at altitudes lower than 600 m, especially in regions with warmer temperatures, even when managed properly. Although Serra et al. (2012) suggested a wider distribution area for *M. capixaba* species, obtained by potential distribution modeling, our sampling efforts reported no occurrence *M. capixaba* in regions north of Santa Tereza (ST), east of Marechal Floriano (MF), south of Vargem Alta (VA), and west of Brejetuba (BJ) (Fig 1).

Climatological stations located in areas where we commonly observed *M. capixaba* had recorded average annual temperatures (Aat) below 19°C in the 30-year historical series (1977–2006). We observed neither the occurrence nor the management of *M. capixaba* colonies in regions of Espírito Santo where the Aat in the historical series was greater than 23°C. Thus, it appears that regions with Aats exceeding 23°C are not favorable for the occurrence or management of this species. Even in regions between 800 m and 1,200 m, but with an Aat of 20–21°C, occurrence of *M. capixaba* has not been verified or it was restricted; it is more common to verify its occurrence in regions at temperatures between 18°C and 20°C (Fig 2B). The observed precipitation in the region of occurrence, using the 30-year historical series, was estimated to be more than 1,200 mm per year, which is higher than the amount of evapotranspiration, thus, the region of occurrence is characterized as a moist environment. According to the climatic classification of Köppen-Geiger, updated by Peel et al. (2007), the prevailing climate in the area estimated for the occurrence of *M. capixaba* is the Tropical Monsoon Climate class "Am." According to this classification, tropical climate regions are characterized by the coldest month of the year with an average temperature greater than or equal to 18°C; absence of seasons with low temperatures (winter seasons); and annual rainfall greater than the annual potential evapotranspiration, with high rates of annual precipitation and precipitation of less than 60 mm in the driest month.

Local vegetation in areas where we sampled *M. capixaba* is almost exclusively Dense Ombrophylous Forest, with some occurrence of Open Ombrophylous Forest, according to the Map of Law Application Area N° 11,428 from 2006 (Atlantic Forest

Table 1. The number of samples and percentage of colonies sampled at each altitude and the minimum and maximum altitudes at which native and managed colonies of *Melipona capixaba* were sampled.

Altitude (m)	Number of colonies	%		Minimum altitude	Maximum altitude
600–700	11	5.67	Native colonies	882 m	1168 m
700–800	16	8.25			
800–900	24	12.37	Managed colonies	612 m	1180 m
900–1000	77	39.69			
1000–1100	61	31.44			
1100–1200	5	2.58			
	194	100			

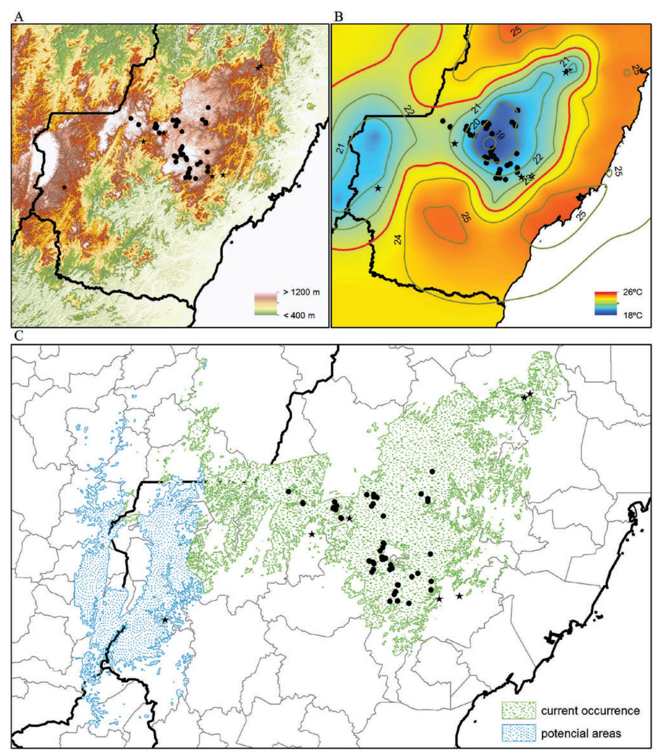

Fig 2. A - Altitude map and occurrence area of *Melipona capixaba*, indicating sampling points and terrain elevation according to the Global Digital Elevation Model - GDEM ASTER (NASA, 2009). B - Spatial distribution map of mean annual temperatures (Tmax), 30-year (1977–2006) time series obtained by interpolation of data from 110 weather stations by using the Kriging spherical model. C - Map of the estimated area of current geographic occurrence (in green) and potential areas (in blue) for species management and conservation, considering altitude, temperature, precipitation, vegetation, native occurrence, and management of species.

Law - IBGE, 2008). In areas where we observed the presence of *M. capixaba*, the Dense Ombrophylous Forest can be classified as Mountain Dense Ombrophylous Forest. The mountain formation is located in high plateaus and/or at ranges of 500 m to 1,500 m, between 16°S and 24°S (IBGE, 1992).

*Current estimated area of occurrence for **M. capixaba** and potential area for the management and conservation of the species*

By comparing the sites where the presence of *M. capixaba* was verified with elevation maps of the spatial distribution of vegetation and annual temperature averages and precipitation, it is possible to delimit an Estimated Area of Natural Occurrence for *M. capixaba*, comprising regions at altitudes between 800 m and 1,200 m, annual temperature averages between 18°C and 23°C, precipitation more than 1,200 mm per year, and with vegetation cover-type Mountain Dense Ombrophylous Forest. The regions with these characteristics are restricted to an area of 3,453 km^2 (Fig 2C, green area).

A potential area for maintenance and occurrence of *M. capixaba* is around the Caparaó National Park, a calculated area of 1,328 km^2 (Fig 2c, blue area). There are no records of *M. capixaba* occurring naturally in the Caparaó region. Although vegetation in this region is classified as Seasonal Semideciduous Forest according to IBGE (2008), a region of Caparaó National Park, especially its eastern portion, has vegetation that is very similar to Mountain Dense Ombrophylous Forest. The proximity of this area to the natural distribution area of *M. capixaba* and its local characteristics suggest that this may be a potential area for species management.

Considering the verified and estimated areas of occurrence, the geographical distribution of species is currently limited to an area of less than 5,000 km^2. Limited areas of distribution suggest that the classification of this species should be changed from its current status of "Vulnerable" VU-B1ab (iii) to "Endangered" EN-B1ab (iii), according to the criteria adopted in the IUCN Red List of Endangered Species (IUCN Red List Categories, 2001). Taxa at a high risk of extinction in the wild are included in the category "Endangered" - EN-B1ab (iii) if they have a geographical distribution of occurrence that is estimated to be below 5,000 km^2, severely fragmented, and with an observed continued decline, inferred or projected in the area, extension and/or quality of habitat. These conditions are consistent with the current situation of *M. capixaba*, justifying its reclassification into the category EN "Endangered."

Serra et al. (2012) suggested that the areas of priority for *M. capixaba* conservation are the following conservation units: Mata das Flores State Park, Forno Grande State Park, Pedra Azul State Park, Caparaó National Park, and Cachoeira do Rio Pardo Forest Reserve. Another area that we consider as an area of priority is the Biological Reserve Augusto Ruschi, which is the largest natural fragment within the estimated area of occurrence with an area of approximately 47 km^2 (4,742 ha). Our sampling could not identify the presence of *M. capixaba* in any of these places, and this was probably because of three main reasons. First, the largest regions in these conservation units are at elevations above 1,200 m, with rocky areas and vegetation on top, which are not good conditions for *M. capixaba* colonies. Second, it is possible that there was local extinction because most of the regions are secondary forests and it is common for people to remove natural colonies for beekeeping. Third, in areas with altitudes between 800 and 1,200 m in the dense ombrophylous forest, sampling of bees is quite difficult because of tree height. Thus, absence of records may be due to sampling limitations. Nevertheless, we still consider some areas of priority as the buffer zones for the conservation unit areas, such as the buffer zone of Forno Grande State Park and Pedra Azul State Park (which is the second largest fragment within the area of occurrence of *M. capixaba*) with an area of approximately 43 km^2 (4,301 ha). This buffer zone is the most promising region in which a program for species management and conservation could be implemented for the maintenance of protected populations (Fig 3B).

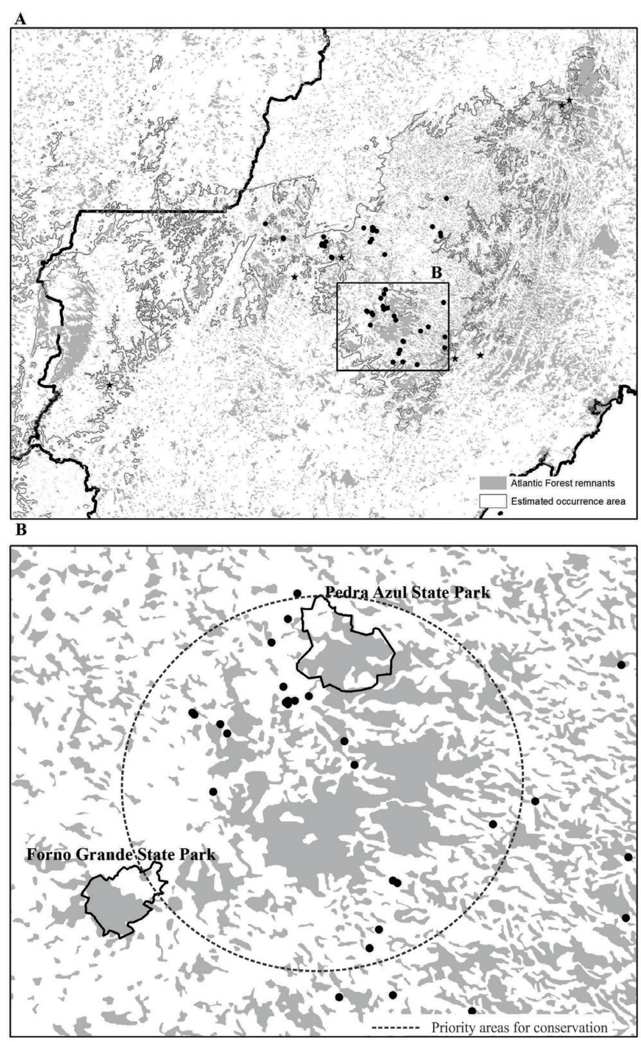

Fig 3. A - Map of Atlantic Forest remnants in the estimated area of *Melipona capixaba* species occurrence. B - Priority areas (hatched line) for the implementation of species conservation and management programs.

Challenges for the conservation of ***M. capixaba***

In the 3,453 km^2 area calculated for species occurrence, only 1,303 km^2 (37.37%) corresponds to the remaining fragments of the Atlantic Forest, forming a mosaic of vegetation (Fig 3a). Of these, 93.5% are remaining fragments smaller than 1 km^2 in size. Habitat fragmentation reduces population sizes and increases isolation of population fragments, resulting in the loss of genetic diversity and consequently increases the risk of species extinction (Frankham et al., 2010). Microsatellite and ISSR markers and mitochondrial haplotypes showed that *M. capixaba* has low genetic variability compared to other insect species (Nogueira et al., 2014).

The remaining forest fragments in the area of *M. capixaba* occurrence are separated by 500 m to more than 5 km in a straight line, which may affect the ability of the species to forage. There is a positive correlation between bee body size and foraging distance (Gathmann & Tscharntke, 2002; Araújo et al., 2004; Greenleaf et al., 2007; Guédot et al., 2009; Zurbuchen et al., 2010; Torné-Noguera et al., 2014). The flight distance of *M. capixaba* has not been determined, but studies of species with similar body sizes suggest that *M. capixaba* can forage in a flight area with a radius of about 2 km. Araújo et al. (2004) estimated that the flight distance of *M. scutellaris* is more than 2 km, and Kuhn-Neto et al. (2009) determined that *M. mandacaia* forages at a distance of 2,100 m.

The main challenge of *M. capixaba* conservation in its natural range is the maintenance of habitats that are capable of providing floral and nesting resources. Considering the size of the remaining fragments and the distance between them, species conservation policies should prioritize not only the maintenance of protected areas but also their connection through ecological corridors that allow adequate foraging in order to support gene flow among isolated colonies. Tewksbury et al. (2002) argued that corridors not only increase the exchange of animals between patches but also facilitate two key animal–plant interactions: pollination and seed dispersal. In addition, these authors suggest that the beneficial effects of the corridors extend beyond the designated area and that increased plant and animal movement through corridors will have positive effects on plant populations and community interactions in fragmented landscapes.

Prospects for the conservation of ***M. capixaba***

One perspective for the conservation of *M. capixaba* is increasing the number of colonies within the area of occurrence through rational rearing of this bee by meliponiculture. Beekeeping of stingless bees in the region of *M. capixaba* occurrence is common among local farmers; however, after inclusion of the species in the List of Threatened Fauna, this practice became prohibited by law. Considering that many colonies of *M. capixaba* lies are maintained by beekeepers, beekeeping can be considered as an alternate, legal way of maintaining *M. capixaba* in its natural habitat for species preservation.

In our view, formation of populations in protected units is the most viable prospect for increasing the current population in natural areas. Reintroduction is a viable option that has been applied for the conservation of several threatened and endangered taxa, and reversal of defaunation is being achieved through the intentional movement of animals to restore populations (Seddon et al., 2014). Winfree (2010) found that the process of bee restoration has been predominantly for agricultural purposes because of the economic importance of maintaining a pollination relationship in agricultural environments. Outside the agricultural context, the primary objective of bee conservation or translocation is improvement of the conservation status of the focal species. From this perspective, it is interesting that species conservation programs take into account the genetic diversity of species and future sustainability of the environment when faced with predicted climate change scenarios.

The forested areas of Pedra Azul and Forno Grande State Park, Biological Reserve Augusto Ruschi, Caparaó National Park, and buffer zones of these conservation units are the major areas for reintroduction of *M. capixaba*, although there are no records of natural colonies in some these sites. In general, choice of the reintroduction area must take into account the known or inferred historical geographical distribution of the focal species or physical evidence of species occurrence. When direct evidence is inadequate to confirm previous occupancy, the existence of a suitable habitat within ecologically appropriate proximity to the proven range may be adequate evidence of previous occupation (IUCN/SSC, 2013). Thomas (2011) argued that there is no need to recreate past ecological communities because of climate change, and, for many species, the only viable option for maintaining the populations of these species in the wild is to translocate them to other locations where the climate is suitable.

Predictions of environmental change in the southeastern portion of the Atlantic Forest indicate a relatively low temperature increase between 0.5°C and 1°C and a rainfall increase of 5–10% by 2040. The temperature will gradually increase by 1.5°C to 2°C and rainfall will increase by 15% to 20% in 2041–2070. By the end of the century (2071–2100), it will become further accentuated with climate patterns between 2.5°C and 3°C increase in temperature and 25–30% increase in rainfall (PBMC, 2014). Considering the current and future climate conditions, the above mentioned places suggested by us for reintroduction should be able to sustain viable populations of *M. capixaba*. They should be able to maintain environmentally and ecologically favorable conditions for the presence of *M. capixaba*, even with the projected temperature increase for the region.

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