

Bot fly parasitism in mantled howler monkeys (*Alouatta palliata*): General patterns and climate influences

Ricardo J. Ortíz-Zárte¹  | Ariadna Rangel-Negrín¹  |
Alejandro Coyohua-Fuentes¹ | Sergio Ibáñez-Bernal²  |
Jurgi Cristóbal-Azkarate³  | Pedro A. D. Dias¹ 

¹Primate Behavioral Ecology Lab, Instituto de Neuro-etología, Universidad Veracruzana, Xalapa, México

²Instituto de Ecología A. C. (INECOL), Red Ambiente y Sustentabilidad, Xalapa, México

³Department of Basic Psychological Processes and their Development, University of the Basque Country, Donostia, España

Correspondence

Ricardo J. Ortíz-Zárte and Pedro A. D. Dias, Primate Behavioral Ecology Lab, Instituto de Neuro-etología, Universidad Veracruzana, Av. Dr. Luis Castelazo Ayala S/N, CP 91190 Xalapa, México.

Email: ricardojesus.ortiz@upaep.mx, pedroaddias@gmail.com and pilantra24@hotmail.com

Funding information

Leakey Foundation; Consejo Nacional de Humanidades, Ciencias y Tecnologías, Grant/Award Number: 1147976

Abstract

Parasitism is a strong selective pressure, and its study is crucial for predicting the persistence of host species. Mantled howler monkeys are infected by the larvae of the bot fly *Cuterebra baeri*. This parasitosis produces myiasis and may have negative impacts on host health, although systematic information on the dynamics of this host-parasite relationship is very limited. Currently, all available information on infection patterns of *C. baeri* comes from a single mantled howler monkey population (Barro Colorado Island, Panama). Therefore, in this study we describe temporal variation in infection patterns for a newly mantled howler monkey population and analyze the relationship between climate and infection likelihood. We assessed the presence of *C. baeri* nodules in 17 adult individuals in Los Tuxtlas for 10 months through direct observation and compiled data on ambient temperature and rainfall. Most subjects had nodules during the study and there were no differences between sexes in the number of nodules. Nodules were usually located in the neck. Prevalence and abundance of nodules peaked thrice during the study (February, April, and September), a pattern that was very similar to that of parasitism intensity (February, April, and August). Incidence closely tracked these peaks, increasing before and decreasing after them. The likelihood of nodule appearance increased when both mean and minimum temperature decreased in the 24–21 prior days to nodule appearance. It also increased with decreased rainfall in the 5–2 prior days to nodule appearance. Although only three of the eight analyzed climate variables had a significant effect on parasitosis, these results suggest that climate may affect pupal development and the access of larvae to hosts. Besides contributing data on *C. baeri* parasitism for a new mantled howler monkey population, our study provides novel information on the influence of environmental factors on the dynamics of host-parasite systems.

KEYWORDS

climate windows, *Cuterebra*, host-parasite dynamics, Los Tuxtlas, oestrids

1 | INTRODUCTION

Parasitism is a symbiotic relationship where one organism (parasite) benefits at the expense of another (host). Proximately, parasites affect hosts through nutrient depletion, tissue damage, and changes in host behavior, which may result in decreased body condition, immunosuppression, and secondary infections (Bush et al., 2001; Sánchez et al., 2018; Sher & Coffman, 1992). Ultimately, parasites play a crucial role in ecological systems by influencing the population dynamics of host species (Goater et al., 2014; Hatcher et al., 2006) through impacts on growth (Gorrell & Schulte-Hostedde, 2008), reproduction (Schwanz, 2008), and survivorship (Robar et al., 2010). Therefore, parasitism is a strong selective pressure, and its study is crucial for predicting the persistence of host species.

Many parasites cannot complete their life cycle without having a parasitic relationship with a host. However, some parasites have free-living states in which they can live without being associated with a host, usually through the releasing eggs or larvae into the environment (Anderson and May, 1979; Roberts et al., 2013). Consequently, the latter are more susceptible to environmental influences, such as climate (Altizer et al., 2006). Humidity and ambient temperature are important factors for the distribution, transmission, and survival of parasite species (Harvell et al., 2002; Heeb et al., 2000; Moyer et al., 2002; Needham & Teel, 1991; Poulin, 2006). For instance, the likelihood of flea infestation of great tit (*Parus major*) nests increases with humidity (Heeb et al., 2000) and the abundance of lice (Insecta: Ischnocera) in several bird species also increases with humidity (Moyer et al., 2002). Regarding temperature, in winter, with lower temperatures, parasitic arthropods become less active, fail to develop, and are more likely to die (Altizer et al., 2006; Eisen

et al., 2016). Thus, climate variation affects the likelihood of parasites associating with a host (Ogden & Lindsay, 2016), which may lead to temporal fluctuations in infection patterns (Oda et al., 2018; Oorebeek & Kleindorfer, 2008, 2009).

The bot fly *Cuterebra baeri* is a host-specific dipteran whose larvae parasitize howler monkeys (*Alouatta* spp.; de Thoisy et al., 2001; Dunn, 1934; Guimarães, 1971; Rondón et al., 2023; Solórzano-García & Pérez-Ponce de León, 2018; Treves & Carlson, 2012; Zeledón et al., 1957). However, parasitosis have also been recorded in a white-fronted capuchin monkey (*Cebus albifrons aequatorialis*; Vilchez-Delgado et al., 2022), monkeys of the genus *Aotus* (Guimarães, 1971; Rondón et al., 2023), and in humans (Fraiha et al., 1984; Guimarães & Coimbra, 1982). *C. baeri* has a life cycle of approximately 96 days (Figure 1). Adult females can mate at 3–5 days of age, lay eggs (probably on leaves: Catts, 1982), and die thereafter (Colwell & Milton, 1998; Milton, 1996). Eggs remain in the substrate for 5 days and both warming and exposure to breath seem to stimulate hatching (Colwell & Milton, 1998; Milton, 1996). Larvae that fail to quickly penetrate a host (<15 min) die (Colwell & Milton, 1998). Once inside the host, the larvae go through three developmental instars for 35–49 days (Colwell & Milton, 1998; Cristóbal-Azkarate et al., 2012). Mature larvae leave the host through a pore, fall to the ground, and burrow into the soil for pupation (Milton, 1996), with flies emerging after 41–49 days (Colwell & Milton, 1998; Milton, 1996).

C. baeri larvae produce myiasis, the infestation of vertebrate animals by dipteran larvae that feed on the host's dead or living tissue, liquid body substances, or ingested food (Zumpt, 1965). Myiasis is reflected in the thoracic region and around the neck of hosts (Catts, 1982), to which howler monkeys mount a strong local

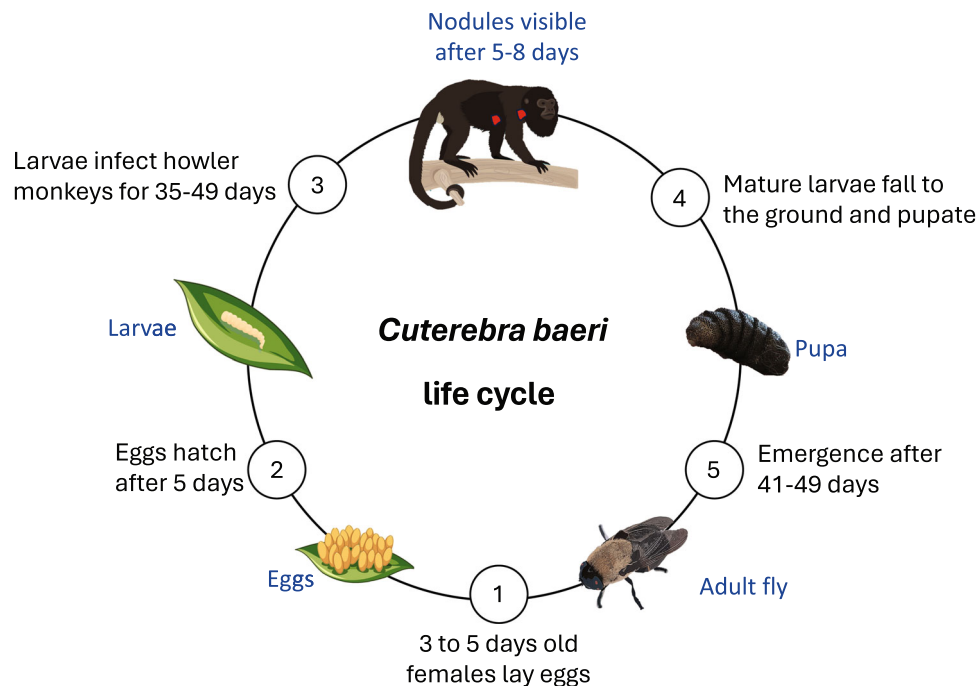


FIGURE 1 The life cycle of the bot fly *Cuterebra baeri*. Data from Colwell and Milton (1998) and Milton (1996).

immune response by encapsulating larvae in conspicuous subdermal nodules (Cristóbal-Azkarate et al., 2012; Hopkins & Milton, 2016; Milton, 1996) that can reach 3.5 cm in diameter (J. Cristóbal-Azkarate, personal observation). As part of the immune response, systemically, howler monkeys synthesize immunoglobulin G antibodies to larval antigens which persist after the parasites leave the host (Baron et al., 1996), although it is unknown for how long they are maintained. This suggests that howler monkeys may acquire resistance to *C. baeri* through multiple exposures, so that parasite numbers are reduced, as is host mortality (Baron et al., 1996). Yet, several parasitized individuals die, either due to the exhaustion of bodily reserves (e.g., fat and albumin) and secondary infections (e.g., bacteria proliferate in empty nodules), or due to the interactions between these and food scarcity (Milton, 1996).

Parasitism of howler monkeys by *C. baeri* has been reported from southern Mexico to Colombia (Cristóbal-Azkarate et al., 2012; Rondón et al., 2023), but infections seem to be absent in several populations (Cristóbal-Azkarate et al., 2012). Additionally, infection patterns vary through time, with one to three peaks observed per year (Milton, 1996). Given the ca. 3-month life cycle of *C. baeri*, this patchy spatial and temporal distribution of infections suggests that the presence and voltinism (i.e., number of generations in a year) may be linked to the effects of environmental factors on bot flies. For instance, it has been suggested that rainfall may have a positive influence on *C. baeri* viability (Milton, 1996). Currently, however, information on infection patterns comes from a single population (Barro Colorado Island, Panama) limiting our understanding of host-parasite dynamics in this system.

This study has two aims. First, we describe for the first time *C. baeri* infection patterns for mantled howler monkeys (*Alouatta palliata*) in Los Tuxtlas (Mexico), including information on the number of individuals infected, number of nodules, location of nodules, sex differences in parasitism, as well as temporal variation in parasitic nodule prevalence, abundance, intensity, and incidence. Second, we examine the effect of climate on parasitization risk by *C. baeri*. Regarding the second aim, we hypothesized that infection patterns of mantled howler monkeys should be conditional on the impact of climate on the free-living stages of the life cycle of *C. baeri*. We predicted that the appearance of parasitic nodules in mantled howler monkeys should depend on temperature and rainfall in the approximately 5 (larval stage), 10 (egg stage), 15 (mating stage), or 16 to 45 (pupal stage) days before the appearance of the first signs of parasitism.

2 | METHODS

2.1 | Study site and subjects

We conducted the study at La Flor de Catemaco, a ca. 100 ha forest fragment located in Los Tuxtlas, Mexico (18°26'43"N, 95°02'49"W). Vegetation at this site is tropical evergreen forest, although in some areas the original understory and forest floor vegetation were

replaced by palm plantations. The climate is hot and humid: during the study monthly mean \pm SD ambient temperature was $24.4 \pm 2.0^\circ\text{C}$ (range = 21.4°C in December– 27.2°C in May; Figure 2) and rainfall was 375.7 ± 206.4 mm (range = 114 mm in March–674 mm in September; total rainfall = 4509 mm). Three climatic seasons occur at Los Tuxtlas: a dry season characterized by high temperatures and low rainfall; a wet season characterized by high temperatures and high rainfall; and a transition season with low to intermediate temperatures and intermediate to low rainfall.

Mantled howler monkeys living at La Flor de Catemaco have been studied since 2002 and are habituated to the presence of researchers (Dias et al., 2023). We studied two groups comprising 17 adults (Group 1: six females and four males; Group 2: four females and three males) that we individually recognized by natural markings in their fur and other physical traits, such as scars, broken fingers, and facial features.

Although infants and juveniles are susceptible to being parasitized by *C. baeri* (Milton, 1996), we did not study them because death and dispersal would make the longitudinal monitoring of infection patterns difficult. Infants are the age group most likely to die (Cristóbal Azkarate et al., 2017; Dias et al., 2023) and juveniles can emigrate from their natal groups when they are completely weaned, which can occur as early as 14 months of age (Balcells & Veá, 2009).

2.2 | *C. baeri* nodule observation

Between July 2022 and April 2023, we conducted observations on the presence of *C. baeri* nodules in study subjects. Observation periods consisted of the following of each group for five consecutive days (mean \pm SD duration of observation periods was 4.8 ± 4.0 days), with a total of 36 periods and 127 observation days conducted during the study. During each visit, we inferred the presence and degree of *C. baeri* infection through detailed observations of each individual performed with Bushnell 10 \times 42 mm binoculars. We noted the individual identity, date, number, and location of the parasitic nodules as well as whether each parasitic nodule was closed, opened, opened without parasite, or absent (i.e., a nodule was not observed where it was previously seen). We recorded changes in the appearance of bot fly nodules through time (i.e., closed to opened to opened without parasite to no-nodule) to determine the duration of each stage.

2.3 | Climate data

We compiled climate data from an automatic meteorologic station located ca. 5 km from the study site and managed by CONAGUA (National Water Commission of Mexico). This station follows the recording standards of the World Meteorological Organization and registers ambient temperature (in $^\circ\text{C}$) and rainfall (in mm) at 10 min intervals. Based on this data we calculated daily minimum, mean, and maximum ambient temperature as well as daily total rainfall.

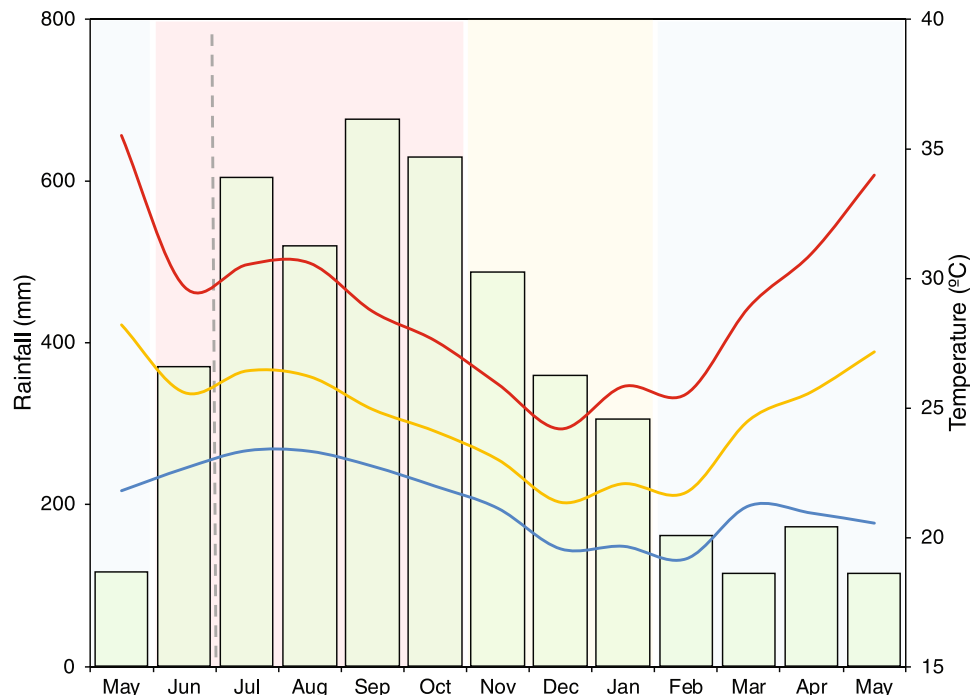


FIGURE 2 Climate during the study: minimum (blue line), mean (orange line), and maximum ambient temperature (red line); rainfall (green bars). Observations of mantled howler monkeys at La Flor de Catemaco (Los Tuxtlas, Mexico) were conducted from July 2022 to April 2023 (months to the right of the dotted vertical line), but climate window analysis considered climate since May 2022. At Los Tuxtlas the dry season elapses between February and May (pale blue shading), the wet season includes the months of June to October (pale red shading), and the transition season occurs from November to January (pale yellow shading).

2.4 | Data organization and analysis

We assessed sex differences in the number of nodules with a chi-square test in which we adjusted expected nodule frequencies by the number of individuals of each sex. We also calculated the mean duration of bot fly nodules (i.e., time from the first observation of a nodule to its disappearance) and the following parasitism measures (Margolis et al., 1982): prevalence (the number of infected individuals divided by the total number of individuals); abundance [the number of nodules divided by the total number of individuals; relative density in Milton (1996)]; intensity (the number of nodules divided by the total number of infected individuals); and incidence (number of individuals with a new nodule divided by individuals without nodules at the beginning of the observation period). These calculations were performed per 5-day observation period (i.e., $n = 36$).

To assess the influence of climate on the likelihood that bot flies parasitize mantled howler monkeys we used Climate Window Analysis implemented with the *climwin* package (Bailey & van de Pol, 2016) in R 4.3.2 (R Core Team, 2024). Climate Window Analysis allows identifying the time at which the influence of a climatic variable on a response variable is the strongest. In our study, we tested the effects of daily minimum, mean, and maximum ambient temperature as well as rainfall on the appearance of bot fly nodules. Given that the life cycle of *C. baeri* from the beginning of pupation to the moment that parasitic nodules become visible elapses ca. 60 days (Figure 1), we explored how the first observation of a given

bot fly nodule was related to the climate during the previous 60 days. We aggregated each climate variable by taking the mean and the variation of all daily values contained within the climatic time window. In *climwin* variation is assessed with the “slope” aggregate statistic, corresponding to the estimated slope of the relationship between the climatic variable and the biological response over the selected window (Bailey & van de Pol, 2016). Therefore, a positive slope suggests an increasing trend, whereas a negative slope indicates a decreasing trend.

For each climate variable and aggregated measure, we created a generalized linear mixed model with a binomial error distribution and a logit link function (i.e., in each observation day nodules were either present or absent; recording of any given botfly nodule between the first and last day of observation were excluded from analysis), with subject identity added as a random factor to account for the repeated sampling of individuals. Before fitting the models, we rescaled each climate predictor. Using an information-theoretic approach, we identified the best supported model, defined as the model minimizing the corrected Akaike information criterion corrected for sample size (AICc; Burnham & Anderson, 2002) compared a null model without climate variables (van de Pol et al., 2016). Given that the number of possible windows in a 60-day period is very large (>1800 combinations), sliding window analysis involves multiple testing and a high likelihood of committing type I statistical errors. To account for these problems, we used randomization tests ($n = 10$ per model) to determine the reliability of the relationship between each

climate variable and bot fly nodule occurrence (i.e., the existence of a true climate signal) with a K-fold cross-validation implemented with *climwin*. These tests yield a statistic (P_c) that allows discriminating true signals from type I and type II errors. Specifically, P_c measures the relative importance of the climate variable in explaining the biological response compared to other potential explanatory variables. In simulation studies, it was established that $P_c < 0.5$ may be used as a cut-off for true climate signals, with lower more conservative values reducing the chance of false positives but simultaneously increasing false negative rates (Bailey & van de Pol, 2016). Therefore, we considered $P_c < 0.5$ as an indicator of a true climate signal for nodule occurrence.

3 | RESULTS

3.1 | General patterns

During the study period, we observed 15 of the 17 adult mantled howler monkeys with at least one bot fly nodule. The two individuals not observed with bot fly nodules were a female and a male. We observed 34 nodules, of which 62% were in the neck region, 26% on the flanks, 6% on the belly, 3% on the chest, and 3% on the head. Seventy-one percent of the bot fly nodules were observed in females (10 individuals) and 29% in males (7 individuals; Table 1), although the difference in the observed nodule frequency was not significant ($\chi^2 = 1.9, p = 0.163$). The mean \pm SD duration of visible bot fly nodules was 30.7 ± 9.1 days (range = 14–50 days) that, if added to 5–8 days, which is the time reported by Colwell and Milton (1998) for the time of infestation to the emergence of nodules containing larvae, yields a mean time of larval development of approximately 38 days.

We calculated the parasitism measures (prevalence, abundance, intensity, and incidence) at the population level, and found three peaks in bot fly nodule prevalence and abundance: February, April, and September (Figure 3a,b). Mean \pm SD parasite prevalence was 14 ± 13 infected individuals (range = 0%–47%) and parasite abundance was 0.2 ± 0.2 bot fly nodules per observed individual (range = 0–0.7). We also observed three peaks in infection intensity: February, April, and August (Figure 3c). Mean intensity was 0.9 ± 0.4 nodules/infected individual (range = 0–1.5). No individuals were parasitized in March and November. There were three periods of increase and two of decrease in incidence: incidence increased in December–January, April, and August–September; and decreased in February–March and September–November (Figure 3d; i.e. incidence increased before and decreased after abundance and intensity peaks).

3.2 | Nodule occurrence and climate

Only three of the eight analyzed climatic factors indicated a true climate signal for nodule occurrence. We found little evidence that mean values of climate variables predicted the occurrence of bot fly nodules in mantled howler monkeys (Table 2, Figure 4), with maybe

TABLE 1 Parasitism attributes per individual mantled howler monkey studied at La Flor de Catemaco (Los Tuxtlas, Mexico), from July 2022 to April 2023.^a

Individual	Sex	Group	#Nodules	Location
MT	Male	1	0	—
MM	Male	1	2	Belly, right/neck, left
ML	Male	1	2	Neck, right/throat
M2	Male	1	1	Neck, left
HF	Female	1	5	Neck, right (3) ^a /belly, right/flank, right
HB	Female	1	3	Flank, left/neck, left/neck, right
H1	Female	1	1	Neck, left
HS	Female	1	1	Neck, left
HN	Female	1	2	Neck, left/neck, right
HSp	Female	1	6	Neck, right (2)/throat (2)/chest, right/flank, left
MJ	Male	2	3	Flank, left/flank, right/neck, right
MP	Male	2	1	Head, left
MCN	Male	2	1	Flank, left
HPG	Female	2	2	Flank, left/flank, right
HPM	Female	2	0	—
HA	Female	2	2	Flank, right/neck, left
HCC	Female	2	2	Neck, right/neck, left

^aNumber of observed nodules.

the exception of a positive effect of daily maximum temperature. However, the best model for maximum daily temperature consisted of a single-day time window and the randomization test equaled the 0.5 P_c threshold. Regarding variation in climate (i.e., 'slope' aggregate measure), the likelihood of nodule occurrence increased with decreasing minimum temperature and mean temperature 24–21 days before observation, and with decreasing rainfall 5–2 days before observation.

4 | DISCUSSION

We aimed at describing the patterns of mantled howler monkey parasitism by *C. baeri* for the first time in Mexico and examining the influence of climate on the occurrence of this parasitism. Most study subjects were infected by *C. baeri* during the study and there were no differences between sexes in the number of observed nodules. Nodules were usually located on the neck. Prevalence and abundance of nodules peaked thrice during the study (February, April, and September), a pattern that was very similar to that of parasitism

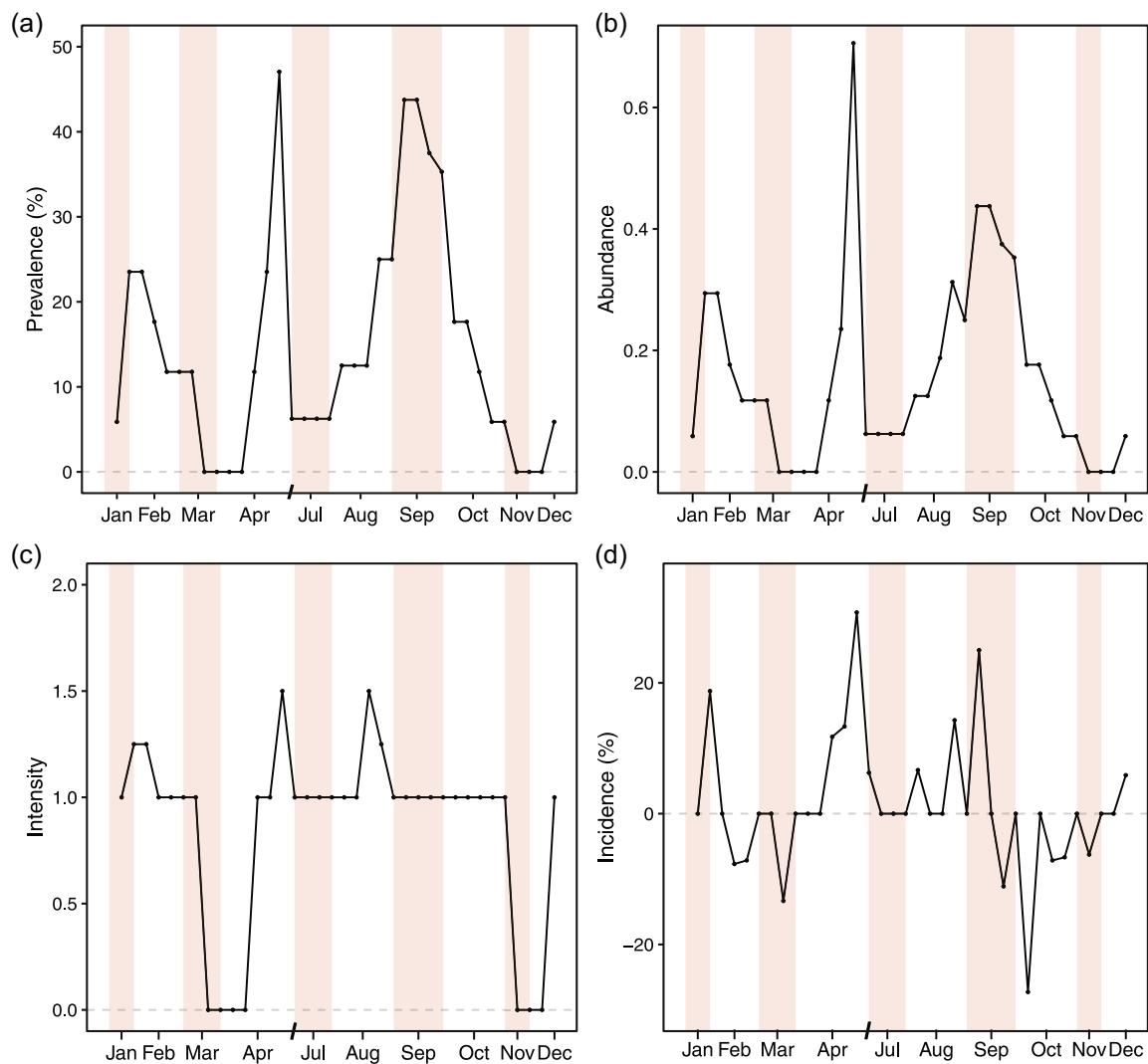


FIGURE 3 Population level parasitism of mantled howler monkeys studied at La Flor de Catemaco (Los Tuxtlas, Mexico) by bot flies (*Cuterebra baeri*): prevalence (a), abundance (b), intensity (c), and incidence (d). For a more intuitive depiction of data, we represent study months in calendar order, although observations were conducted from July 2022 to April 2023. The x-axis scale break indicates that no data were collected in May and June.

intensity (February, April, and August). Parasitism incidence closely tracked these peaks (i.e., incidence increased before peaks and decreased after them). We found some support for the hypothesis that the occurrence of parasitism is linked to the influence of climate on the free-living stages of the life cycle of *C. baeri*. Specifically, the likelihood of nodule appearance increased with decreasing mean and minimum temperature in the 24–21 prior days and with decreasing rainfall 5–2 days before nodule appearance. Therefore, our study contributes data on *C. baeri* parasitism for a new mantled howler monkey population as well as new knowledge of the influence of environmental factors on the dynamics of this host-parasite system.

This study represents the first report outside Barro Colorado, Panama, describing temporal changes in parasitosis levels of howler monkeys by bot flies, and the first time statistical techniques have been used to analyze the effect of climate on this parasitosis. Our results align with previous observations that *C. baeri* nodules tend to

concentrate in the upper body of mantled howler monkeys, and more specifically in the neck (Calderón-Arguedas et al., 2004; Cristóbal-Azkarate et al., 2012; Milton, 1996). Given that the infection mechanism and internal migration patterns of larvae are currently unknown, it is unclear why most nodules are in these body areas.

Bot fly larvae prevalence, abundance, and intensity in our study groups were lower than those reported for other groups in Los Tuxtlas (Cristóbal-Azkarate et al., 2012), the Caribbean coast of Costa Rica (Calderón-Arguedas et al., 2004), and Barro Colorado, Panama (Milton, 1996; Table 3). The values reported by Cristóbal-Azkarate et al. (2012) and Calderón-Arguedas et al. (2004) represent a snapshot in time and do not provide any ecological context, limiting our ability to interpret the observed similarities and differences. On the other hand, the study by Milton (1996) followed 8–10 groups for 36 months and included monthly estimates of prevalence, infection intensity, and monitoring of howler monkey mortality, although it is

unclear if the same individuals were observed through time. In this sense, the author argues that high dietary stress associated with low fruit consumption (the main source of readily available energy for mantled howler monkeys) negatively affects the immune system of

TABLE 2 Results of sliding window analyses of the influence of climatic factors on bot fly infestation in mantled howler monkeys living at La Flor de Catemaco (Los Tuxtlas, Mexico) studied from July 2022 to April 2023.

Predictor	Statistic	Estimate \pm SE	Δ AIC _c	Best window (days)	P _c
Minimum temperature	Mean	0.5 \pm 0.2	-4.6	19-18	0.52
	Slope	-2.4 \pm 0.6	-13.5	24-21	0.15
Mean temperature	Mean	0.6 \pm 0.3	-5.6	33-33	0.53
	Slope	-2.2 \pm 0.6	-10.3	24-21	0.36
Maximum temperature	Mean	0.7 \pm 0.3	-7.3	4-4	0.50
	Slope	-1.2 \pm 0.2	-9.3	4-2	0.55
Rainfall	Mean	0.5 \pm 0.2	-6.3	3-2	0.56
	Slope	-1.2 \pm 0.3	-9.7	5-2	0.48

the howler monkeys, facilitating the establishment of high larval burdens. Compared to Barro Colorado, howler monkeys at La Flor de Catemaco spend considerably more time feeding on fruit (42% vs 59%, respectively; Milton, 1980; Rangel-Negrin et al., 2021). This difference could be related to the lower density of howler monkeys in La Flor de Catemaco (0.4 individuals/ha: Dias et al., 2023; Barro Colorado Island, Panama, 0.8 individuals/ha: Milton, 1996) and the absence of competing spider monkeys (*Ateles geoffroyi*), a fruit specialist, in our study area. Yet, without information on nutrient acquisition, physical condition, and individual immunological status it is currently impossible to establish whether differences between populations are linked to variation in resource availability or other factors, including overall climate patterns. Future research focused on the intersection of the physiology, behavior, and ecology of mantled howler monkeys related to *C. baeri* infection should allow examining these proposals.

The uneven time intervals between the three peaks in parasitism measures support the proposal that infection patterns do not directly correspond to the number of possible *C. baeri* generations in a year, given its life cycle. Temporal variation in host immunity related to access to food resources (mentioned above) could account for this variation, but climate window analyses also suggests that these peaks could be linked to the influence of climate on certain stages of the parasite life cycle. If *C. baeri* from Los Tuxtlas and Barro Colorado

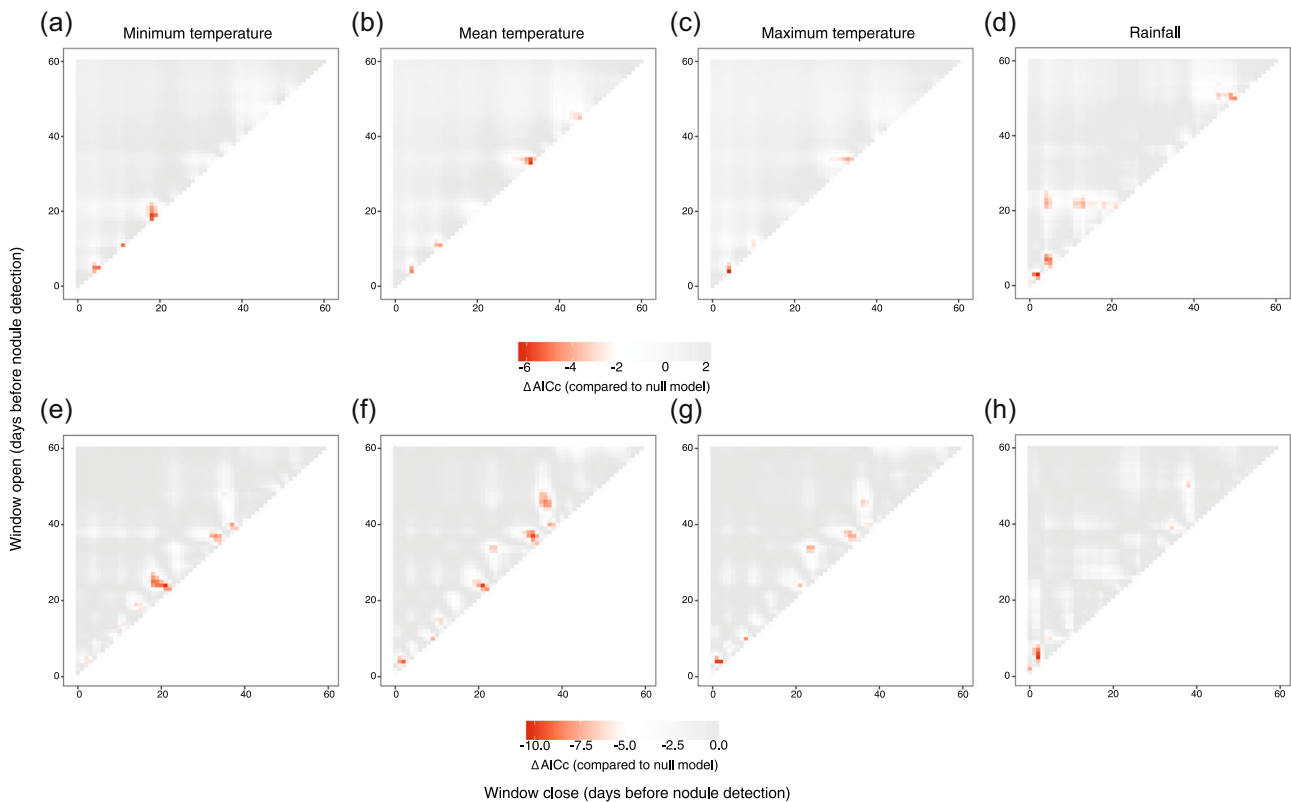


FIGURE 4 AICc values of climate models relating the occurrence of bot fly (*Cuterebra baeri*) nodules in mantled howler monkeys living at La Flor de Catemaco (Los Tuxtlas, Mexico) with climate from July 2022 to April 2023. Panels (a-d) depict model results based on mean climate values whereas panels (e-h) pertain to models based on variation (i.e., slope) in climate variables.

TABLE 3 Measures of bot fly parasitism for different populations of mantled howler monkeys.

Measure	Site			
	Barro Colorado Island (Panamá)	Caribbean coast of Costa Rica	Los Tuxtlas (México)	La Flor de Catemaco (México)
Prevalence	60	29	28	14.6
Abundance ^a	1.9	0.7	1.1	0.2
Intensity	2.8	2.75	-	0.9
Incidence	-	-	-	1.3
Sample size	55-106	28	46	17
Study	Milton (1996)	Calderón-Arguedas et al. (2004)	Cristóbal-Azkarate et al. (2012)	This study

^aRelative density in Milton (1996).

have a similar life cycle (Milton, 1996), then our results indicate that decreasing minimum and mean ambient temperatures during pupation (i.e., 24–21 days before bot fly nodule detection) have a positive effect on *C. baeri*, as inferred by increased infection likelihood of mantled howler monkeys. These results are consistent with evidence of temperature effects on oestrid viability. For instance, when exposed to persistent high temperatures, the pupae of sheep bot flies (*Oestrus ovis*) lose weight, resulting in lightweight flies that may have decreased fitness (Cepeda-Palacios & Scholl, 2000). Similarly, the eclosion of reindeer bot flies (*Hypoderma tarandi* and *Cephenemyia trompe*) occurs later in cold than in hot summers, reducing the number of days in which adults can fly, and thus, mate (Nilssen, 1997). This evidence, however, does not help explaining why decreasing temperature over a specific period favors pupae development. Thus, the specific impact of variation in ambient temperature on *C. baeri* remains to be determined by studies focusing on the physiology and development of this oestrid.

As infection peaks encompassed cold (January, February) and hot (March, April; August, September) months, no common pattern seems to exist across temperature time windows. In this sense, even if decreases in ambient temperature have an overall positive effect on pupae, the moment at which these changes occur may further influence the pace of pupal development, as reported for other oestrid species (Breev et al., 1980; Nilssen, 1997). Specifically, following the September prevalence and abundance peak, there was a sustained drop in temperatures until the next peak, which occurred 4 months later in February; whereas temperatures increased gradually from February to the April peak, i.e. a 2-month lag. If shorter pupation is associated with decreased temperature in warmer periods of the year (dry season), an additional peak in parasitism could occur in May and June. Unfortunately, we could not sample mantled howler monkeys in those months, so the impact of decreasing temperatures on both the duration and success of pupal development and, ultimately, infection patterns of mantled howler monkeys, remains to be examined.

The likelihood of infection increased when rainfall decreased 3–5 days before nodule emergence, a time window that corresponds to the egg and larval stages of *C. baeri* life cycle. As *C. baeri* eggs have

structural traits to resist water (Colwell et al., 1999), it is possible that rainfall mostly affects recently hatched larvae, especially because these seem to be fragile (i.e., they have 15 min to enter a host before dying: Colwell & Milton, 1998). Downpours, which are common at Los Tuxtlas (Soto & Gama, 1997) and occurred in all study months, could wash larvae from vegetation (the putative oviposition substrate of *C. baeri*: Colwell & Milton, 1998), precluding infection (Stromberg, 1997). If this is the case, rainfall would affect *C. baeri* parasitism directly (i.e., mechanical actions on larvae) rather than indirectly via its impact on soil moisture, as previously speculated (Milton, 1996). In contrast with previous studies, we correlated climate and infection patterns at a fine temporal scale (i.e., 0–60 days) and, using climate window analysis, we explored the relationships between climatic variables and parasitism with minimal a priori assumptions in terms of the timeframe in which those relationships should occur (i.e., definition of the range of dates in which analyses should be performed: Bailey & van de Pol, 2016). We propose that future assessments of the ecology of mantled howler monkey infection by *C. baeri* should base on similar methods instead of using broad classifications of climate (i.e., climate 'seasons': Baron et al., 1996; Calderón-Arguedas et al., 2004; Milton, 1996). Again, given the limited scope of our study (10-month observation of 17 subjects living in a single site), the patterns identified here must be confirmed by further research.

Predictions for future climatic conditions suggest that maximum temperature of the warmest month, minimum temperature of the coldest month, and the annual range of temperature will increase by 2050 at Los Tuxtlas; and annual precipitation is predicted to decrease in most of Mexico's natural protected areas (Esperon-Rodriguez et al., 2019). Climatic changes like these would probably affect the free-living stages of the life cycle of *C. baeri*. This may be reflected in the species voltinism and its parasitic dynamics with mantled howler monkeys. A possible alteration of the equilibrium between parasite and host could imply fitness consequences and affect the persistence of both species.

We conclude that the overall patterns of *C. baeri* infection of mantled howler monkeys converge with previous research, although we detected some variation in parasitism measures that could be linked to

differences between populations in immunological, demographic, and ecological factors. We also found some support for the hypothesis that infection patterns are influenced by environmental factors, as infection likelihood was linked to decreases in ambient temperature and rainfall, a possible consequence of the impact of these factors on pupal development and access of larvae to hosts. Finally, we have no evidence of adult mantled howler monkey mortality associated with *C. baeri* infection in La Flor de Catemaco, neither during this study nor in ca. 20 years of population monitoring (Dias et al., 2023). This contrasts with reports from the only other site for which we previously had long-term information on this parasitosis (Milton, 1996) and warrants future inter-population comparative research.

AUTHOR CONTRIBUTIONS

Jurgi Cristóbal-Azkarate and Pedro A. D. Dias originally formulated the idea. Ricardo J. Ortiz-Zárate, Jurgi Cristóbal-Azkarate, and Pedro A. D. Dias developed methodology. Ricardo J. Ortiz-Zárate and Alejandro Coyohua-Fuentes conducted fieldwork. Ricardo J. Ortiz-Zárate, Ariadna Rangel-Negrín, and Pedro A. D. Dias performed statistical analyses. All authors wrote the manuscript.

ACKNOWLEDGMENTS

We thank D. Canales Espinosa, Laura Johnson, and Jamie Whewell for their support during fieldwork and Ing. J. L. Ponce Puente for allowing the work at La Flor de Catemaco S.A. We also thank A. Di Fiore and two anonymous reviewers for comments that greatly improved previous versions of the manuscript. The study was financed by Consejo Nacional de Ciencia y Tecnología (CONACyT grant 1147976), Posgrado en Neuroetología, Instituto de Neuro-etología (Universidad Veracruzana), and the Leakey Foundation. A. Rangel-Negrín and Pedro A. D. Dias thank Mariana and Fernando for continued inspiration to understand primate behavior.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data from this study are available from the corresponding authors upon reasonable request.

ETHICS STATEMENT

Our research protocols were approved by the Secretaría de Medio Ambiente y Recursos Naturales (permit SGPA/DGVS/04015/21) and complied to the legal requirements of the Mexican law. The research was noninvasive and adhered to the American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates.

ORCID

Ricardo J. Ortiz-Zárate  <http://orcid.org/0000-0003-2623-4633>
 Ariadna Rangel-Negrín  <https://orcid.org/0000-0003-3539-3652>
 Sergio Ibáñez-Bernal  <https://orcid.org/0000-0002-3182-6134>
 Jurgi Cristóbal-Azkarate  <https://orcid.org/0000-0002-2799-8638>
 Pedro A. D. Dias  <http://orcid.org/0000-0002-2919-6479>

REFERENCES

- Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., & Rohani, P. (2006). Seasonality and the dynamics of infectious diseases. *Ecology Letters*, 9, 467–484. <https://doi.org/10.1111/j.1461-0248.2005.00879.x>
- Anderson, R. M., & May, R. M. (1979). Population biology of infectious diseases: Part I. *Nature*, 280, 361–367. <https://doi.org/10.1038/280361a0>
- Bailey, L. D., & van de Pol, M. (2016). Climwin: An R toolbox for climate window analysis. *PLoS One*, 11, e0167980. <https://doi.org/10.1371/journal.pone.0167980>
- Balcells, C. D., & Veà, J. J. V. (2009). Developmental stages in the howler monkey, subspecies *Alouatta palliata mexicana*: a new classification using age-sex categories. *Neotropical Primates*, 16, 1–8. <https://doi.org/10.1896/044.016.0101>
- Baron, R. W., Colwell, D. D., & Milton, K. (1996). Antibody immunoglobulin G (IgG) response to *Alouattamyia baeri* (Diptera: Cuterebridae) parasitism of howler monkeys, *Alouatta palliata*, in Panama. *Journal of Medical Entomology*, 33, 946–951. <https://doi.org/10.1093/jmedent/33.6.946>
- Breev, K. A., Zagretidinov, R. G., & Minár, J. (1980). Influence of constant and variable temperatures on pupal development of the sheep bot fly (*Oestrus ovis* L.). *Folia Parasitologica*, 27, 359–365.
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference*. Springer.
- Bush, A. O., Fernández, J. C., Esch, G. W., & Seed, J. R. (2001). *Parasitism: The diversity and ecology of animal parasites*. Cambridge University Press.
- Calderón-Arguedas, O., Troyo, A., Solano, M. E., Sánchez, R., Chinchilla, M., & Gutiérrez-Espeleta, G. A. (2004). Infección por larvas de *Alouattamyia baeri* (Diptera: Cuterebridae) en monos aulladores, *Alouatta palliata* (Primates: Cebidae) de la Costa Caribe de Costa Rica. *Neotropical Primates*, 12, 21–24.
- Catts, E. P. (1982). Biology of new world bot flies: Cuterebridae. *Annual Review of Entomology*, 27, 313–338. <https://doi.org/10.1146/annurev.en.27.010182.001525>
- Cepeda-Palacios, R., & Scholl, P. J. (2000). Factors affecting the larvipositional activity of *Oestrus ovis* gravid females (Diptera: Oestridae). *Veterinary Parasitology*, 91, 93–105. [https://doi.org/10.1016/S0304-4017\(00\)00265-X](https://doi.org/10.1016/S0304-4017(00)00265-X)
- Colwell, D. D., Baird, C. R., Lee, B., & Milton, K. (1999). Scanning electron microscopy and comparative morphometrics of eggs from six bot fly species (Diptera: Oestridae). *Journal of Medical Entomology*, 36, 803–810. <https://doi.org/10.1093/jmedent/36.6.803>
- Colwell, D. D., & Milton, K. (1998). Development of *Alouattamyia baeri* (Diptera: Oestridae) from howler monkeys (Primates: Cebidae) on Barro Colorado island, Panama. *Journal of Medical Entomology*, 35, 674–680. <https://doi.org/10.1093/jmedent/35.5.674>
- Cristóbal-Azkarate, J., Dunn, J. C., Domingo Balcells, C., & Veà Baró, J. (2017). A demographic history of a population of howler monkeys (*Alouatta palliata*) living in a fragmented landscape in Mexico. *PeerJ*, 5, e3547. <https://doi.org/10.7717/peerj.3547>
- Cristóbal-Azkarate, J., Colwell, D. D., Kenny, D., Solórzano, B., Shedden, A., Cassaigne, I., & Luna, E. R. (2012). First report of bot fly (*Cuterebra baeri*) infestation in howler monkeys (*Alouatta palliata*) from Mexico. *Journal of Wildlife Diseases*, 48, 822–825. <https://doi.org/10.7589/0090-3558-48.3.822>
- Dias, P. A. D., Coyohua-Fuentes, A., Canales-Espinosa, D., & Rangel-Negrín, A. (2023). Demography and life-history parameters of mantled Howler monkeys at La flor de Catemaco: 20 years post-translocation. *Primates*, 64, 143–152. <https://doi.org/10.1007/s10329-022-01030-z>
- Dunn, L. H. (1934). Entomological investigations in the Chiriqui region of Panama. *Psyche: A Journal of Entomology*, 41, 166–183. <https://doi.org/10.1155/1934/21960>

- Eisen, R. J., Eisen, L., Ogden, N. H., & Beard, C. B. (2016). Linkages of weather and climate with *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae), enzootic transmission of *Borrelia burgdorferi*, and Lyme disease in North America. *Journal of Medical Entomology*, 53, 250–261. <https://doi.org/10.1093/jme/tjv199>
- Esperon-Rodríguez, M., Beaumont, L. J., Lenoir, J., Baumgartner, J. B., McGowan, J., Correa-Metrio, A., & Camac, J. S. (2019). Climate change threatens the most biodiverse regions of Mexico. *Biological Conservation*, 240:108215. <https://doi.org/10.1016/j.biocon.2019.108215>
- Fraiha, H., Lopes Chaves, L. C., Borges, I. C., & Barros de Freitas, R. (1984). Miíases humanas na Amazônia—III: miíase pulmonar por *Alouattomyia baeri* (Shannon & Greene, 1926) (Diptera, Cuterebridae). *Revista da Fundação Serviço de Saúde Pública*, 29, 63–68.
- Goater, T. M., Goater, C. P., & Esch, G. W. (2014). *Parasitism: The diversity and ecology of animal parasites* (2da ed). Cambridge University Press.
- Gorrell, J. C., & Schulte-Hostedde, A. I. (2008). Patterns of parasitism and body size in red squirrels (*Tamiasciurus hudsonicus*). *Canadian Journal of Zoology*, 86, 99–107. <https://doi.org/10.1139/Z07-123>
- Guimarães, J. H. (1971). Notes on the hosts of Neotropical Cuterebrini (Diptera, Cuterebridae), with new records from Brazil. *Papéis Avulsos de Zoologia*, 25, 89–94.
- Guimarães, J. H., & Coimbra, Jr., C. E. A. (1982). Miíase humana por *Alouattomyia baeri* (Shannon & Greene) (Diptera, Cuterebridae). Comunicação de dois casos na região norte do Brasil. *Revista Brasileira de Zoologia*, 1, 35–39. <https://doi.org/10.1590/S0101-81751982000100005>
- Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science*, 296, 2158–2162. <https://doi.org/10.1126/science.1063699>
- Hatcher, M. J., Dick, J. T. A., & Dunn, A. M. (2006). How parasites affect interactions between competitors and predators. *Ecology Letters*, 9, 1253–1271. <https://doi.org/10.1111/j.1461-0248.2006.00964.x>
- Heeb, P., Kölliker, M., & Richner, H. (2000). Brid-ectoparasite interactions, nest humidity, and ectoparasite community structure. *Ecology*, 81, 958–968. [https://doi.org/10.1890/0012-9658\(2000\)081\[0958:beinha\]2.0.co;2](https://doi.org/10.1890/0012-9658(2000)081[0958:beinha]2.0.co;2)
- Hopkins, M. E., & Milton, K. (2016). Adverse effects of ball-chain radiocollars on female mantled howlers (*Alouatta palliata*) in Panama. *International Journal of Primatology*, 37, 213–224. <https://doi.org/10.1007/s10764-016-9896-y>
- Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M., & Schad, G. A. (1982). The use of ecological terms in parasitology (Report of an Ad Hoc Committee of the American Society of Parasitologists). *The Journal of Parasitology*, 68, 131–133. <https://doi.org/10.2307/3281335>
- Milton, K. (1980). *The foraging strategy of howler monkeys: A study of primate economics*. Columbia University Press.
- Milton, K. (1996). Effects of bot fly (*Alouattomyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata*) population in Panama. *Journal of Zoology*, 239, 39–63. <https://doi.org/10.1111/j.1469-7998.1996.tb05435.x>
- Moyer, B. R., Drown, D. M., & Clayton, D. H. (2002). Low humidity reduces ectoparasite pressure: Implications for host life history evolution. *Oikos*, 97, 223–228. <https://doi.org/10.1034/j.1600-0706.2002.970208.x>
- Needham, G. R., & Teel, P. D. (1991). Off-host physiological ecology of Ixodid ticks. *Annual Review of Entomology*, 36, 659–681. <https://doi.org/10.1146/annurev.en.36.010191.003303>
- Nilssen, A. C. (1997). Effect of temperature on pupal development and eclosion dates in the reindeer oestrids *Hypoderma tarandi* and *Cephenemyia trompe* (Diptera: Oestridae). *Environmental Entomology*, 26, 296–306. <https://doi.org/10.1093/ee/26.2.296>
- Oda, F. H., Kitagawa, C., Noronha, J. D. C., Rodrigues, D. D. J., Martins, T. F., Valadão, M. C., Carvalho, L. M., & Campos, A. K. (2018). Amblyomma tick species infesting amphibians and reptiles in the seasonally dry Amazon forest, with new host records for *Amblyomma rotundatum* (Acari: Ixodida: Ixodidae). *Systematic and Applied Acarology*, 23, 387–392. <https://doi.org/10.11158/saa.23.2.14>
- Ogden, N. H., & Lindsay, L. R. (2016). Effects of climate and climate change on vectors and vector-borne diseases: Ticks are different. *Trends In Parasitology*, 32, 646–656. <https://doi.org/10.1016/j.pt.2016.04.015>
- Oorebeek, M., & Kleindorfer, S. (2008). Climate or host availability: What determines the seasonal abundance of ticks? *Parasitology Research*, 103, 871–875. <https://doi.org/10.1007/s00436-008-1071-8>
- Oorebeek, M., & Kleindorfer, S. (2009). The prevalence and intensity of tick infestation in passerines from South Australia. *Emu - Austral Ornithology*, 109, 121–125. <https://doi.org/10.1071/MU08052>
- van de Pol, M., Bailey, L. D., McLean, N., Rijdsdijk, L., Lawson, C. R., & Brouwer, L. (2016). Identifying the best climatic predictors in ecology and evolution. *Methods in Ecology and Evolution*, 7, 1246–1257. <https://doi.org/10.1111/2041-210X.12590>
- Poulin, R. (2006). Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. *Parasitology*, 132, 143–151. <https://doi.org/10.1017/S0031182005008693>
- R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. R Core Team. <https://www.R-project.org/>
- Rangel-Negrín, A., Coyohua-Fuentes, A., de la Torre Herrera, A., Cano-Huertes, B., Reynoso-Cruz, E., Ceccarelli, E., Gómez-Espinosa, E. E., Chavira-Ramírez, D. R., Moreno-Espinoza, D. E., Canales-Espinosa, D., Maya-Lastra, N., Cruz-Miros, P., Cañadas-Santiago, S., Garau, S., & Dias, P. A. D. (2021). Female reproductive energetics in mantled howler monkeys (*Alouatta palliata*): A follow-up study. *American Journal of Physical Anthropology*, 174, 396–406. <https://doi.org/10.1002/ajpa.24222>
- Robar, N., Burness, G., & Murray, D. L. (2010). Tropics, trophics and taxonomy: The determinants of parasite-associated host mortality. *Oikos*, 119, 1273–1280. <https://doi.org/10.1111/j.1600-0706.2009.18292.x>
- Roberts, L. S., Janovy, Jr., J., & Nadler, S. (2013). *Gerald D. Schmidt & Larry S. Roberts' foundations of parasitology* (9th ed). McGraw-Hill.
- Rondón, S., Cavallero, S., Link, A., De Meo, M., González, C., & D'Amelio, S. (2023). *Cuterebra baeri* infecting grey-legged night monkeys (*Aotus griseimembra*) and red howler monkeys (*Alouatta seniculus*) in Colombia. *Journal of Wildlife Diseases*, 59, 181–185. <https://doi.org/10.7589/JWD-D-22-00062>
- Sánchez, C. A., Becker, D. J., Teitelbaum, C. S., Barriga, P., Brown, L. M., Majewska, A. A., Hall, R. J., & Altizer, S. (2018). On the relationship between body condition and parasite infection in wildlife: A review and meta-analysis. *Ecology Letters*, 21, 1869–1884. <https://doi.org/10.1111/ele.13160>
- Schwanz, L. E. (2008). Chronic parasitic infection alters reproductive output in deer mice. *Behavioral Ecology and Sociobiology*, 62, 1351–1358. <https://doi.org/10.1007/s00265-008-0563-y>
- Sher, A., & Coffman, R. L. (1992). Regulation of immunity to parasites by T-cells and T-cell derived cytokines. *Annual Review of Immunology*, 10, 385–409. <https://doi.org/10.1146/annurev.iy.10.040192.002125>
- Solórzano-García, B., & Pérez-Ponce de León, G. (2018). Parasites of neotropical primates: A review. *International Journal of Primatology*, 39, 155–182. <https://doi.org/10.1007/s10764-018-0031-0>
- Soto, M., & Gama, L. (1997). Climas. In R. Vogt, E. González-Soriano, & R. Dirzo (Eds.), *Historia natural de Los Tuxtlas* (pp. 7–25). UNAM.
- Stromberg, B. E. (1997). Environmental factors influencing transmission. *Veterinary Parasitology*, 72, 247–264. [https://doi.org/10.1016/s0304-4017\(97\)00100-3](https://doi.org/10.1016/s0304-4017(97)00100-3)
- de Thoisy, B., Vogel, I., Reynes, J.-M., Pouliquen, J.-F., Carme, B., Kazanji, M., & Vié, J.-C. (2001). Health evaluation of translocated

- free-ranging primates in French Guiana. *American Journal of Primatology*, 54, 1–16. <https://doi.org/10.1002/ajp.1008>
- Treves, A., & Carlson, A. E. (2012). Botfly parasitism and tourism on the endangered black howler monkey of Belize. *Journal of Medical Primatology*, 41, 284–287. <https://doi.org/10.1111/j.1600-0684.2012.00549.x>
- Vilchez-Delgado, F., Ramírez-Montano, L. F., Merino-Merino, X., Ojeda-Juárez, R. R., Espinoza-Román, M. H., & Duarte-Quiroga, A. (2022). Botfly parasitism on two primates of the Cebidae family in Peru. *Journal of Wildlife Diseases*, 58, 415–420. <https://doi.org/10.7589/JWD-D-21-00175>
- Zeledón, R., Otto Jiménez, Q., & Brenes, R. R. (1957). *Cuterebra baeri* Shannon y Greene, 1926 en el mono aullador de Costa Rica. *Revista de biología tropical*, 5, 129–134.
- Zumpt, F. (1965). *Myasis in man and animals in the old world: A textbook for physicians, veterinarians, and zoologists*. Butterworths.

How to cite this article: Ortíz-Zárate, R. J., Rangel-Negrín, A., Coyohua-Fuentes, A., Ibáñez-Bernal, S., Cristóbal-Azkarate, J., & Dias, P. A. D. (2024). Bot fly parasitism in mantled howler monkeys (*Alouatta palliata*): General patterns and climate influences. *American Journal of Primatology*, e23680. <https://doi.org/10.1002/ajp.23680>