ARTICLE

Life post-death: Colonization of a bat carcass by *Microcerella halli* (Engel, 1931) (Diptera: Sarcophagidae) in a Neotropical cave

Simão Dias Vasconcelos[1]; Eder Barbier[2] & Taciano Moura Barbosa[3]

[1] Universidade Federal de Pernambuco (UFPE), Centro de Biociências (CB), Departamento de Zoologia, Laboratório de Insetos de Importância Forense. Recife, PE, Brasil.
ORCID: https://orcid.org/0000-0002-8722-0836. E-mail: simao.vasconcelosfo@ufpe.br

[2] Universidade Federal de Pernambuco (UFPE), Centro de Biociências (CB), Departamento de Zoologia, Laboratório de Ciências Aplicadas à Conservação da Biodiversidade. Recife, PE, Brasil.
ORCID: https://orcid.org/0000-0001-5068-7048. E-mail: barbier.eder@gmail.com

[3] Universidade Federal do Rio Grande do Norte (UFRN), Centro de Biociências (CB), Departamento de Microbiologia e Parasitologia (DMP), Laboratório de Insetos e Vetores (LIVE). Natal, RN, Brasil.
ORCID: https://orcid.org/0000-0002-5495-258X. E-mail: tacianomoura7@gmail.com (corresponding author)

Abstract. Caves are habitats characterized by low light or total darkness, whose fauna is largely composed of animals that adapt to dark environments, such as bats and many arthropods. Here, we present the first record of bat carcass colonization by Diptera larvae in a cave in the Neotropics. Twenty-one adult specimens of *Microcerella halli* (Sarcophagidae), seven males and 14 females, emerged from larvae collected in a carcass of *Pteronotus gymnonotus* (Mormoopidae) found in a twilight zone, about 15 m from the cave entrance. The mean time between carcass collection and adult emergence was 17.99 ± 0.44 days. As colonization by *M. halli* only occurs after death, we estimated the mean duration of the pupal stage – by recording daily pupation and emergence data – and attempted a preliminary estimate of the post-mortem interval, which indicated that larviposition occurred at least 24 h before carcass collection. Furthermore, we discussed the fact that *M. halli* females can find and colonize carcasses in low light environments, reinforcing the forensic potential of the species.

Keywords. *Microcerella;* Mormoopid bats; Post-mortem interval; Flesh flies.

INTRODUCTION

Caves are oligotrophic environments where the limited resource availability demands unique specialization for the adaptation and survival of biological entities (Schneider *et al.,* 2011). Caves represent a peculiar habitat characterized by a combination of low light or total darkness, relatively stable temperature and humidity, and low availability of trophic resources (Culver & Pipan, 2009). These stressful factors induce physiological, metabolic, morphological and behavioural adaptations in the hypogean fauna (Howarth & Moldovan, 2018), including insects.

Characterization of insect diversity in caves has been frequently based on three approaches: i) descriptions of invertebrate communities associated with bat guano (Ferreira & Martins, 1999; Gnaspini & Trajano, 2000); ii) inventories of food items consumed by insectivorous cave animals (Souza-Silva & Ferreira, 2014) and iii) studies on bat ectoparasitism by streblid flies (Barbier *et al.,*

2018). The occurrence of necrophagous insects and their role in carrion decomposition in caves, on the other hand, has been virtually ignored.

The input of organic matter provided by dead animals depends largely upon the action of sarcosaprophagous Diptera, especially species of the families Calliphoridae, Sarcophagidae and Muscidae. By feeding directly on carcasses, dipteran larvae help in the decomposition of carcasses – and thus, in the cycling of nutrients. Shifts in time of initial colonization of carrion by Diptera can impact nutrient recycling efficiency and, subsequently, the rate of nutrient introduction into the ecosystem (Heo *et al.,* 2019).

Carrion decomposition is expected to occur more slowly in environments under limited insect access, such as caves. Therefore, it is important to understand how nutrients are incorporated into cave deposits – and which organisms are involved in this process. However, little is known about the ability of scavenger species to effectively penetrate the dark zone in search of substrate for their

larvae. Terrell-Nield & MacDonald (1997) reported dipterans (*e.g.,* Calliphoridae and Phoridae) as components of the invertebrate community in dead animal remains in caves in England.

Interestingly, the presence and temporal succession of insect species on carcasses (and cadavers) provide reliable tools for the estimation of the minimum post-mortem interval (minPMI), a key foundation of forensic entomology. Considering the importance of caves as threatened habitats, and the growing interest on forensic entomology techniques applied to wild animals, we describe in this article a pioneering record of bat carcass colonization by Diptera larvae in a cave. We also attempt to validate the estimation of post-mortem interval applied to crimes against wildlife.

MATERIAL AND METHODS

The bat carcass was found on November 19th, 2021 in the 'Furna do Morcego' cave (08°34'14.1"S, 37°22'55.4"W; 557 m a.s.l.; Fig. 1a). The cave is located in an environmental protection area in the *Caatinga* domain, a type of seasonally dry tropical forest, which occupies most of north-eastern Brazil. The climate in the region is hot semi-arid (*BSh,* Köpper-Geiger), with an average annual temperature of 23°C. This sandstone cave has a single entrance, about 44 m of horizontal projection and can be subdivided into two main chambers, with the bat colonies concentrated in the deepest chamber (Fig. 1b).

The cave shelters much higher bat populations than the average for caves in the Neotropics, reaching more than 70,000 individuals (Leal & Bernard, 2021). Five bat species have already been recorded in this cave, of which *Pteronotus gymnonotus* (Wagner, 1843) and *P. personatus* (Wagner, 1843) (Mormoopidae) are the dominant species (Leal & Bernard, 2021).

The bat carcass was identified as *P. gymnonotus,* based on the striking external features, such as the wing membranes being fixed on the midline of the dorsum of its body and its large size compared to other naked-backed *Pteronotus* (Pavan & Tavares, 2020). *Pteronotus gymnonotus* is distributed across most of the Neotropical mainland, ranging from southeastern Mexico through Central and South America to north-eastern Bolivia and central Brazil (Pavan & Tavares, 2020). Adults typically weigh 12 to 18 g and are insectivorous. Like other mormoopids, *P. gymnonotus* usually forms large colonies roosting preferentially in hot and humid caves (Pavan, 2019).

The bat carcass was found hanging on the cave wall, at a distance of about 15 m from the entrance – in the twilight zone – and approximately 2 m above the ground (Fig. 1c). The site is about 20 m from where the colony is sheltered and has characteristics of surface-subterranean interface, but does not receive direct sunlight. The bat was transferred to the laboratory in a 500 mL screened container containing vermiculite to allow pupation of the insects. At the moment of transfer, we observed second and third instar Sarcophagidae (Diptera) larvae.

The carcass was kept under laboratory conditions at 24°C, photoperiod of 12:12 (L:D) and was observed twice a day. The temperature and photoperiod conditions were controlled with air conditioning and a timer to determine the 12L:12D cycle. This cycle period was used based on life tables available in the literature. As soon as specimens in the pre-pupal stage were visualized, they were transferred to individual containers containing vermiculite until all larvae had pupated. Typically, necrophagous larvae tend to abandon the carcass to pupate in the surrounding soil, so we observed the prepupae located distant from the carcass. From then on, the emergence of adults was observed twice a day, until the emergence of all individuals. Adults were identified following the taxonomical key of Vairo *et al.* (2011) and Vairo *et al.* (2015). By registering daily data on pupation and emergence, we estimated the mean duration of the pupal stage and attempted a preliminary estimation of the post-mortem interval. Adult insects were properly pinned, labeled and deposited at the Entomological Collection of UFPE, Brazil.

RESULTS

Twenty-one specimens were collected from the carcass (Fig. 1d) and all completed their life cycle in the laboratory. Seven male and 14 female adults emerged. The colonizing species was identified as *Microcerella halli* (Engel, 1931) (Diptera: Sarcophagidae).

In our study, pre-pupae were observed as early as four days after finding the carcass (Fig. 2). All pupae were formed 24 h after the insects had reached the pre-pupal stage. The mean duration of pupal stage was 12.48 ± 0.51 days. The earliest emergence of adults occurred 17 days after carcass collection (Fig. 2). The mean time from collection of the carcass until the emergence of adult was 17.99 ± 0.44 days.

DISCUSSION

The genus *Microcerella* Macquart is restricted to the Neotropical region and has 78 species distributed in South America (Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Peru and Venezuela) (Pape, 1996; Mulieri *et al.,* 2015). *Microcerella* species feed on decomposing animal matter, such as beef, chicken viscera, squid, snake, rat and pig carcasses, and also on human corpses (Madeira-Ott *et al.,* 2022). *Microcerella halli* has been recorded in association with ephemeral decomposing resources such as carcasses of lizards (Madeira-Ott *et al.,* 2022) and snakes (Moretti *et al.,* 2009).

Reproductive traits of *M. halli* are typical of most Sarcophagidae, that is, gravid females lay first instar larvae (not eggs) on the substrate. We ruled out the possibility of larviposition in the living animal because *M. halli* has not been reported to cause myiasis, and we did not observe tissue rupture in the bat body or orifices resulting from the larvae exit.
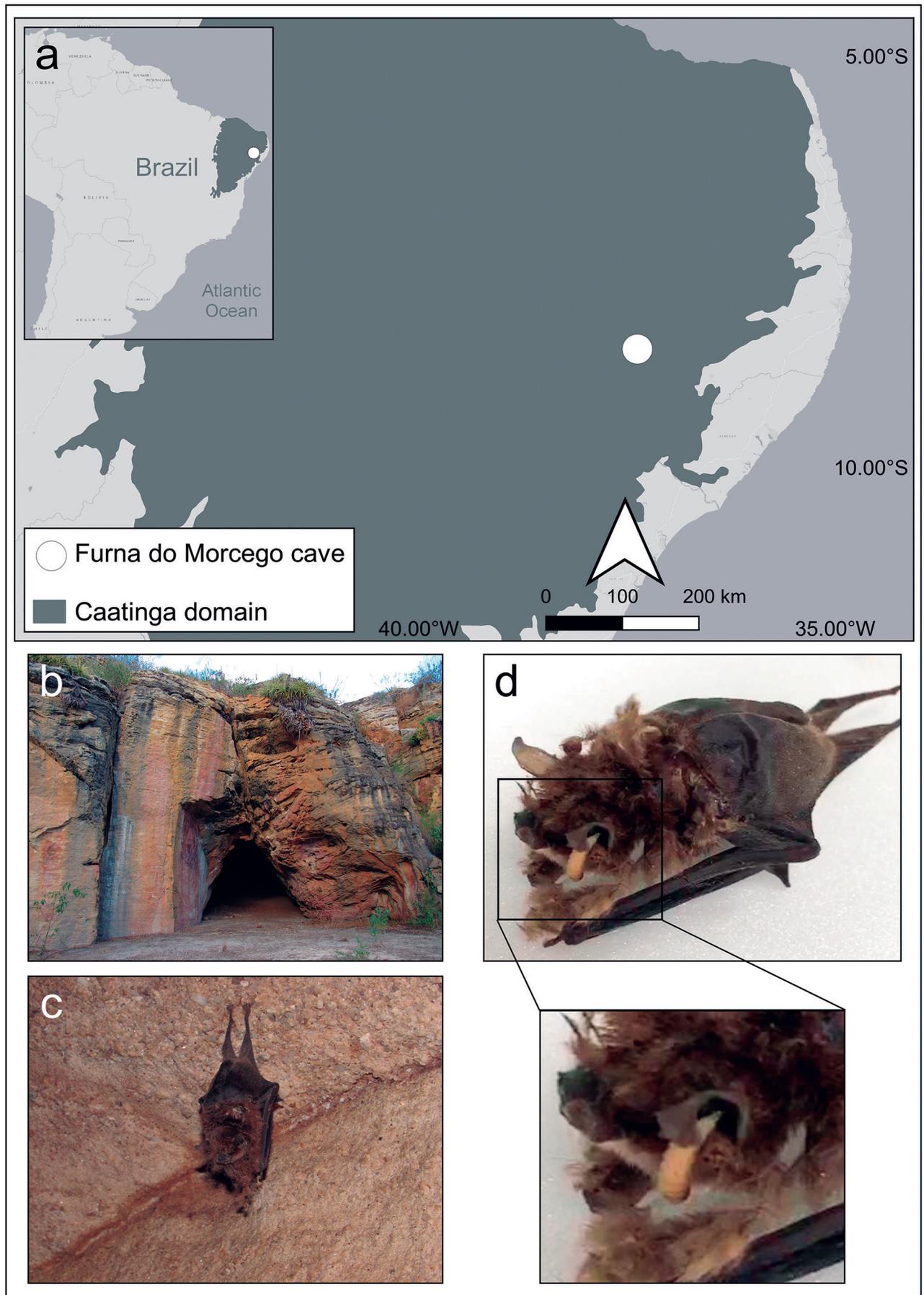
Figure 1. (A) Map showing the location of the cave where the bat carcass was found. (B) View of the entrance of the 'Furna do Morcego' cave. (C) Bat (Mormoopidae: *Pteronotus gymnonotus*) carcass hanging from the cave wall. (D) Detail of carcass colonized by *Microcerella halli* (Diptera: Sarcophagidae) larvae. The shaded area corresponds to the *Caatinga* (seasonally dry tropical forest).
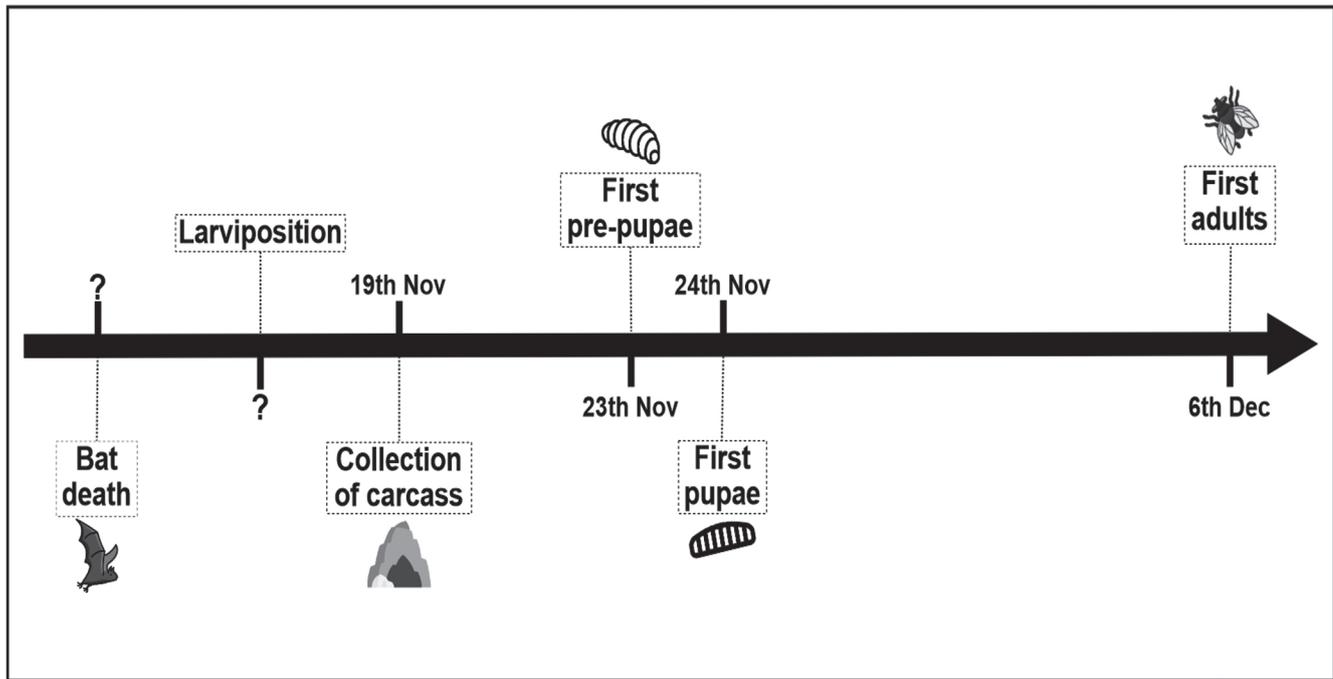
Figure 2. Timeline of bat carcass colonization, depicting the data required for the estimation of minPMI. Icons made by AmethystDesign, Culmbio, Freepik, and Nadiinko from https://www.flaticon.com.

Due to their unique conditions, bat caves supply an unpredictable supply of dead organic matter to necrophagous insects – but only those with higher ability to penetrate twilight/dark zones will outdo competitors in carcass colonization. Sick bats decrease social contact and tend to move away from their colony, probably to minimize the risk of infection to conspecifics (Ripperger *et al.,* 2020). Colony segregation could increase the probability of sick bats dying in places more accessible to flies such as near the cave entrance, increasing the likelihood of colonization by Sarcophagidae, Calliphoridae, and Muscidae.

Cave openings function as a transition between the external environment – of variable rainfall, temperature and relative humidity – to the interior of the cave, where abiotic conditions are more constant. The twilight zones of caves may represent ecotonal areas that harbour a mixed community of surface and subsurface species. Areas close to the entrance may present structural, biological or physical modifications, creating a transition zone between the epigean and hypogean systems (Prous *et al.,* 2004). We believe that interactions between bats (crepuscular or nocturnal) and flies (mostly diurnal) may be more frequent than documented in the literature. For instance, analysis of bat stomach contents revealed a diverse diet that included typically diurnal necrophagous flies, such as blowflies (Calliphoridae), flesh flies (Sarcophagidae), and houseflies (Muscidae) (Rydell *et al.,* 2016). Considering that some bat species can forage at dusk – while necrophagous flies may still be active – the likelihood of bat-insect encounters can not be dismissed.

Caves can be regarded as one of the least explored environment types on Earth (Mammola *et al.,* 2019) and are subject to complex ecological dynamics unlike those at surface communities. So far, data on carrion colonization in caves are mostly anecdotal, due to logistic limita-

tions that include difficulties in performing experiments in the dark and the need for approval by environmental agencies. This is the case of Brazil, which has about 22,800 registered caves (CECAV, 2022) protected by environmental laws.

Nevertheless, necrophagous dipterans have been sparsely registered in caves. *Calliphora vicina* (Calliphoridae) and *Megaselia* spp. (Phoridae) colonized rat carcasses exposed in a cave in England, and the abundance decreased proportionally to the increase in cave depth (Terrell-Nield & MacDonald, 1997). *Calliphora vicina* (Calliphoridae) was shown to be able to lay eggs on a human cadaver in a 10-m-deep cave in a forest in the Swiss Jura mountains at an altitude of 1,260 m (Faucherre *et al.,* 1999). Differently, *C. vicina* was registered only sporadically and at the entrance zones of caves located in the Nearctic Region (Reeves & McCreadie, 2001).

Sarcophagidae, on the other hand, have hardly been registered in caves. For instance, in a survey on 23 caves in Brazil, 21 Diptera families were registered – without occurrence of Sarcophagidae (Ferreira & Martins, 1999). *Microcerella halli,* in particular, has not been detected in caves.

The relevance of *M. halli* in forensic entomology has strengthened in recent years, as it has been recorded colonizing human corpses in indoor environments, for which its development data were used to estimate the minPMI (Vairo *et al.,* 2017). We compared our data with Nassu *et al.* (2014), to attempt at an estimation of the minPMI of the animal. Considering that our samples were at late second/early third instar (visual inspection), we calculated, retroactively, the time elapsed between death and emergence of adults. To achieve that, it was necessary to sum up the time spent at first and second instars (as the species are larviparous).

Our results agree with Nassu *et al.* (2014), in which, under laboratory conditions, the duration of the pupal stage was 14 days at 25°C and 12 days when kept at 30°C. Given the slight difference of temperature in the cave entrance (*ca.* 30°C) and the lab (constant at 24°C), we used a combination of temperatures to estimate the minPMI, and minor variations can be expected. Based on Nassu *et al.* (2014), the cycle from 1st instar until adult is 21.8 days (at 25°C), and 18.8 days (at 30°C). Our data fit accurately in between these values, given that the environment/laboratory temperature was also between those limits. Based on instar duration recorded by Nassu *et al.* (2014) (first + second instar is 0.8 day at 25°C, and 1.34 day at 30°C), we conclude that colonization occurred at least one day prior to collection. Because colonization by *M. halli* only occurs after death, we deduce, with some degree of certainty, that death happened, on or before Nov 18th 2021. This is a minimum estimate; *M. halli* females may have taken some additional time to locate and larviposit on the carcass.

Our findings contribute to expand the knowledge on the behaviour and type of environment occupied by *M. halli*. From an applied standpoint, most attempts of estimation of PMI rely on the assumption that female dipterans require sunlight prior to oviposition (Greenberg, 1990). We document here that *M. halli* females can find (and lay their larvae on) carcasses in dimly lit areas. Considering that homicides frequently occur at night, we call the attention to *M. halli* as a forensically relevant species for investigating animal (including human) killings.

REFERENCES

Barbier, E.; Hintze, F.; Jardelino, A.C. & Bernard, E. 2018. First record of the bat ectoparasitic fly *Strebla proxima* Wenzel, 1976 (Diptera: Streblidae) from Brazil. *Entomological News,* 127(4): 369-374. https://doi.org/10.3157/021.127.0409.

Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV). 2022. Cadastro Nacional de Informações Espeleológicas – CANIE. https://www.icmbio.gov.br/cecav/canie.html. Access: 25/02/2022.

Culver, D.C. & Pipan, T. 2009. *The biology of caves and other subterranean habitats.* New York, Oxford University Press.

Faucherre, J.; Cherix, D. & Wyss, C. 1999. Behavior of *Calliphora vicina* (Diptera, Calliphoridae) under extreme conditions. *Journal of Insect Behavior,* 12(5): 687-690. https://doi.org/10.1023/A:1020931804597.

Ferreira, R.L. & Martins, R.P. 1999. Trophic structure and natural history of bat guano invertebrate communities, with special reference to Brazilian caves. *Tropical Zoology,* 12(2): 231-252. https://doi.org/10.1080/03946975.1999.10539391.

Gnaspini, P. & Trajano, E. 2000. Guano communities in tropical caves. p. 251-268. *In:* Wilkins, H.; Culver, D.C. & Humphreys, W.F. (Eds.). *Subterranean ecosystems.* Amsterdam, Elsevier Press.

Greenberg, B. 1990. Nocturnal oviposition behavior of blow flies (Diptera: Calliphoridae). *Journal of Medical Entomology,* 27(5): 807-810. https://doi.org/10.1093/jmedent/27.5.807.

Heo, C.C.; Teel, P.D.; Banfield, M.M. & Tomberlin, J.K. 2019. Soil arthropod community responses to carrion with delayed insect access. *Food Webs,* 20: e00118. https://doi.org/10.1016/j.fooweb.2019.e00118.

Howarth, F.G. & Moldovan, O.T. 2018. The ecological classification of cave animals and their adaptations. *In:* Moldovan, O.; Kováč, Ľ. & Halse, S. (Eds.). *Cave ecology: ecological studies (analysis and synthesis).* Cham, Springer. p. 41-67. https://doi.org/10.1007/978-3-319-98852-8_4.

Leal, E.B.S. & Bernard, E. 2021. Morcegos cavernícolas do carste arenítico do Parque Nacional do Catimbau, Nordeste do Brasil. *Mastozoología Neotropical,* 28: e0608. https://doi.org/10.31687/saremMN.21.28.2.0.08.e0608.

Madeira-Ott, T.; Souza, C.M.; Bunde, P.R.S.; Ries, A.C.R.; Blochtein, B. & Thyssen, P.J. 2022. Forensically relevant flesh flies (Diptera, Sarcophagidae, Sarcophaginae) of Southern Brazil. *Journal of Medical Entomology,* 59(2): 488-507. https://doi.org/10.1093/jme/tjab210.

Mammola, S.; Cardoso, P.; Culver, D.C.; Deharveng, L.; Ferreira, R.L.; Fišer, C.; Galassi, D.M.P.; Griebler, C.; Halse, S.; Humphreys, W.F.; Isaia, M.; Malard, F.; Martinez, A.; Moldovan, O.T.; Niemiller, M.L.; Pavlek, M.; Reboleira, A.S.P.S.; Souza-Silva, M.; Teeling, E.C.; Wynne, J.J. & Zagmajster, M. 2019. Scientists' warning on the conservation of subterranean ecosystems. *BioScience,* 69(8): 641-650. https://doi.org/10.1093/biosci/biz064.

Moretti, T.C.; Allegretti, S.M.; Mello-Patiu, C.A.; Tognolo, A.M.; Ribeiro, O.B. & Solis, D.S. 2009. Occurrence of *Microcerella halli* (Engel) (Diptera, Sarcophagidae) in snake carrion in southeastern Brazil. *Revista Brasileira de Entomologia,* 53(2): 318-320. https://doi.org/10.1590/S0085-56262009000200018.

Mulieri, P.R.; Mariluis, J.C.; Patitucci, L.D. & Olea, M.S. 2015. The Sarcophaginae (Diptera: Sarcophagidae) of Southern South America. I. The species of *Microcerella* Macquart from the Patagonian Region. *Zootaxa,* 3933: 1-88. https://doi.org/10.11646/zootaxa.3933.1.1.

Nassu, M.P.; Thyssen, P.J. & Linhares, A.X. 2014. Developmental rate of immatures of two fly species of forensic importance: *Sarcophaga (Liopygia) ruficornis* and *Microcerella halli* (Diptera: Sarcophagidae). *Parasitology Research,* 113: 217-222. https://doi.org/10.1007/s00436-013-3646-2.

Pape, T. 1996. Catalogue of the Sarcophagidae of the world (Insecta: Diptera). *Memoirs on Entomology International,* 8: 1-558.

Pavan, A.C. 2019. Family Mormoopidae (ghost-faced bats, naked-backed bats and mustached bats). *In:* Wilson, D.E. & Mittermeier, R.A. (Eds.). *Handbook of the Mammals of the World, Vol. 9. Bats.* Barcelona,Lynx Edicions.

Pavan, A.C. & Tavares, V.C. 2020. *Pteronotus gymnonotus* (Chiroptera: Mormoopidae). *Mammalian Species,* 42: 40-48. https://doi.org/10.1093/mspecies/seaa003.

Prous, X.; Ferreira, R.L. & Martins, R.P. 2004. Ecotone delimitation: epigean-hypogean transition in cave ecosystems. *Austral Ecology,* 29(4): 374-382. https://doi.org/10.1111/j.1442-9993.2004.01373.x.

Reeves, W.K. & McCreadie, J.W. 2001. Population ecology of cavernicoles associated with carrion in caves of Georgia, USA. *Journal of Entomological Science,* 36(3): 305-311. https://doi.org/10.18474/0749-8004-36.3.305.

Ripperger, S.P.; Stockmaier, S. & Carter, G.G. 2020. Tracking sickness effects on social encounters via continuous proximity sensing in wild vampire bats. *Behavioral Ecology,* 31(6): 1296-1302. https://doi.org/10.1093/beheco/araa111.

Rydell, J.; Bogdanowicz, W.; Boonmanc, A.; Petterssond, S.; Suchecka, E. & Pomorski, J.J. 2016. Bats may eat diurnal flies that rest on wind turbines. *Mammalian Biology,* 81(3): 331-339. https://doi.org/10.1016/j.mambio.2016.01.005.

Schneider, K.; Christman, M.C. & Fagan, W.F. 2011. The influence of resource subsidies on cave invertebrates: results from an ecosystem-level manipulation experiment. *Ecology,* 92(3): 765-776. https://doi.org/10.1890/10-0157.1.

Souza-Silva, M. & Ferreira, R.L. 2014. *Loxosceles* spiders (Araneae: Sicariidae) preying on invertebrates in Brazilian caves. *Speleobiology Notes,* 6: 27-32. https://doi.org/10.5563/spbn.v6i0.46.

Terrell-Nield, C. & MacDonald, J. 1997. The effects of decomposing animal remains on cave invertebrate communities. *Cave and Karst Science,* 2: 53-67.

Vairo, K.P.; Caneparo, M.F.C.; Corrêa, R.C.; Preti, D. & Moura, M.O. 2017. Can Sarcophagidae (Diptera) be the most important entomological evidence at a death scene? *Microcerella halli* as a forensic indicator. *Revista Brasileira de Entomologia,* 61(4): 275-276. https://doi.org/10.1016/j.rbe.2017.06.004.

Vairo, K.P.; Mello-Patiu, C.A. & Carvalho, C.J.B. 2011. Pictorial identification key for species of Sarcophagidae (Diptera) of potential forensic importance in southern Brazil. *Revista Brasileira de Entomologia,* 55(3): 333-34. https://doi.org/10.1590/S0085-56262011005000033.

Vairo, K.P.; Moura, M.O & Mello-Patiu, C.A. 2015. Comparative morphology and identification key for females of nine Sarcophagidae species (Diptera) with forensic importance in Southern Brazil. *Revista Brasileira de Entomologia,* 59(3): 177-187. https://doi.org/10.1016/j.rbe.2015.06.003.