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Ecologia de flebotomíneos (Diptera: Psychodidae) coletados em cavernas do estado de Minas Gerais, Brasil

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Introdução Geral

Os flebotomíneos (Diptera: Psychodidae) são dípteros que em todo mundo são conhecidas, aproximadamente 900 espécies, das quais, mais de 500 estão presentes na região Neotropical e destas, mais de 260 já foram registradas no Brasil (Galati 2003a, Ready 2013, Andrade et al. 2014). No Brasil estes insetos estão presentes em todo o território nacional onde aproximadamente 40 espécies são comprovadas ou suspeitas de transmitirem *Leishmania* (Rangel & Lainson 2009), o agente causador das leishmanioses em humanos e outros mamíferos (Forattini 1973, Young & Duncan 1994).

O gênero *Leishmania* (Kinetoplastida: Trypanosomatidae) infecta uma ampla variedade de vertebrados mamíferos (domésticos e selvagens): roedores, canídeos, edentados (tatu, preguiça, tamanduá), marsupiais (gambás), primatas incluindo humanos (Deane & Deane 1954, Lainson et al. 1985, Shaw 2003). O gênero é subdividido em dois subgêneros, *Viannia* e *Leishmania*, (Lainson and Shaw 1987) e compreende aproximadamente 30 espécies, das quais cerca de 20 são patogênicas para a espécie humana (Lainson & Shaw 1998, Ashford 2000, Desjeux 2004, Ready 2013). Entre as parasitoses humanas, as leishmanioses ocupam um lugar de destaque, devido à sua importância médica e econômica sendo um dos maiores problemas de saúde pública (WHO 1990, Barral et al. 1991).

As leishmanioses compreendem um espectro de doenças infecciosas, consideradas zoonoses (Rotureau 2006). As leishmanioses podem ser divididas em dois grupos principais: 1- Leishmaniose Visceral (LV) - conhecida como calazar, causada pelas espécies do subgênero *Leishmania* (*Leishmania*), sendo representada no Brasil pela espécie *Le.* (*Leishmania*) *infantum* (Magill 2000) e 2- Leishmaniose Tegumentar Americana (LTA), do subgênero *Leishmania* (*Leishmania*), sendo representada no Brasil pela espécie *Le.* (*Leishmania*) *amazonensis* e do subgênero *Leishmania*

(*Viannia*), sendo representada no Brasil pelas espécies *Le.* (*Viannia*) *braziliensis*, *Le.* (*Viannia*) *guyanensis*, *Le.* (*Viannia*) *lainsoni*, *Le.* (*Viannia*) *naiffi*, *Le.* (*Viannia*) *lindenbergi* e *Le.* (*Viannia*) *shawi* (Grimaldi & Tesh 1993, Cunningham 2002, Ashford 2000).

A LV é uma zoonose de evolução crônica de grande importância epidemiológica nas Américas, sobretudo no Brasil, devido a sua alta incidência, ampla distribuição geográfica, podendo apresentar formas graves que levam a morte, se não tratadas adequadamente (Gontijo & Melo 2004). Os sintomas mais comuns da LV humana são fraqueza, perda de peso, febre intermitente, anemia, anorexia, pancitopenia e esplenomegalia com ou sem hepatomegalia (WHO 2010).

As infecções por LTA se manifestam frequentemente em áreas expostas do corpo como face, membros e pescoço. No local da picada surgem lesões cutâneas que podem evoluir para diversas formas: cutânea localizada, disseminada, difusa e mucosa (Nadim Faghih 1968, Amato 2003).

A transmissão da infecção para os vertebrados ocorre por meio da picada de fêmeas de flebotomíneos pertencentes a diversos gêneros da subfamília Phlebotominae infectadas com o parasito (Galati 2003).

Os flebotomíneos adultos, machos e fêmeas, alimentam-se de sucos vegetais, néctar de flores, frutos, soluções açucaradas e de sangue, estando este último hábito restrito às fêmeas (Deane & Deane 1957, Forattini 1960, Sherlock & Sherlock 1972, Alexander & Usma 1994). Os carboidratos são utilizados como fonte de energia, necessária para o metabolismo geral do inseto, servindo para as atividades de voo, corte, cópula e dispersão e, no caso das fêmeas o sangue serve para a maturação de seus ovários (Sherlock 2003). Esses insetos podem ser encontrados em ambientes urbanos, rurais e silvestres, incluindo as cavernas (Galati et al. 2003a, b; Alves et al. 2008, Alves et al. 2011, Campos et al. 2013) e suas formas imaturas se desenvolvem em solo úmido, protegido da incidência direta da luz, do vento e rico em matéria orgânica (Forattini 1973).

Os flebotomíneos apresentam atividades crepusculares e noturnas, embora algumas espécies se mostram ativas, sob determinadas condições ambientais durante o dia (Teodoro et al. 1993, Young & Duncan 1994, Sherlock 2003), como observado em diferentes ambientes cavernícolas (Guernaoui et al. 2006, Galati et al. 2010, Alves et al. 2011, Fuenzalida et al. 2011, Polseela et al. 2011, Carvalho et al. 2012).

As cavernas são consideradas ambientes com pequena variação de temperatura e umidade no seu interior, ausência de luz nas áreas mais afastadas da entrada e condições oligotróficas (Poulson & White 1969, Trajano & Gnaspini-Netto 1991). São habitats únicos habitados por uma biota diversificada e singular (Poulson & White 1969). De acordo com Holsinger & Culver (1988), os organismos cavernícolas podem ser classificados em três categorias: troglóbios (os restritos às cavernas e por isso tendem a apresentar diversos tipos de especializações morfológicas, fisiológicas e comportamentais), troglófilos (os adaptados às cavernas, mas podem completar seu ciclo de vida dentro ou fora delas) e trogloxenos (os que utilizam regularmente as cavernas como abrigo, refúgio, local de alimentação ou reprodução).

Esses ambientes, embora aparentemente inóspitos, podem abrigar muitas espécies de vertebrados e invertebrados, incluindo os flebotomíneos (Ferreira & Horta 2001, Galati et al. 2003a, 2003b, 2003c; Alves et al. 2008, Ferreira et a. 2009, Carvalho et al. 2011, 2012, 2013, Matavelli et al. 2015, Saraiva et al. 2015).

Estudos realizados dentro de cavernas têm mostrado resultados interessantes, tais como, registros de novas espécies de flebotomíneos (Galati et al. 2003a, b), diversidade desses insetos em cavernas iguais ou maiores do que o encontrada na floresta e composição de espécies distinta entre caverna e seu entorno (Galati et al. 2003c, 2006, 2010; Alves et al. 2008, Carvalho et al. 2013).

O estudo englobou cavernas e seus respectivos entornos de quatro localidades distintas no estado de Minas Gerais: Parque Estadual Serra do Rola Moça, Parque Estadual Serra do Ouro Branco, Moeda Sul e o município de Pains.

O Parque Estadual Serra do Rola Moça, Parque Estadual Serra do Ouro Branco e Moeda Sul estão localizados na região do Quadrilátero Ferrífero. O Quadrilátero Ferrífero é uma região rica em campos ferruginosos “canga” e está localizado dentro de duas das maiores bacias hidrográficas do Brasil, Rio Doce e Rio São Francisco. Uma região com grande quantidade de cavidades naturais cadastradas (Do Carmo et al. 2011, CECAV 2016) inseridas numa zona de transição de Cerrado e Mata Atlântica, rico em campos ferruginosos e de altitude além das áreas de Capoeira (formações secundárias) (Jacobi & Do Carmo 2008, IEF 2016). Esta região apresenta altos níveis de biodiversidade e endemismo, ocorrência de relativamente grandes áreas naturais, presença de bacias hidrográficas relevantes para o fornecimento de água para um dos maiores centros urbanos do Brasil, e sofre com pressões antrópicas associadas principalmente com atividades de mineração, exploração imobiliária e urbanização (Fernandes et al. 2014, Sonter et al. 2014).

O município de Pains está localizado no centro-oeste de Minas Gerais, distante 230 km de Belo Horizonte. A região faz parte de um complexo que contempla a existência de um grande número de cavidades naturais. As cavernas utilizadas neste estudo estão inseridas em áreas remanescentes do bioma do Cerrado. Essa região localiza-se entre a bacia hidrográfica do Alto São Francisco e a bacia hidrográfica do Rio Grande (SEE 2016). A área estudada sofre com os impactos antrópicos da mineração, calcinação, agricultura.

O estudo se propôs em responder questões sobre os padrões que determinam a distribuição da fauna de flebotomíneos dentro e fora dos ambientes cavernícolas e também compreender a atividade diária desses insetos, o que pode ajudar a esclarecer questões relativas ao período de maior risco de transmissão de *Leishmania* entre os flebotomíneos e os hospedeiros vertebrados que visitam ou vivem nessas cavernas, incluindo o homem. Esta tese é apresentada na forma de dois capítulos: 1) Diferença de fotoperíodo na riqueza e abundância de flebotomíneos (Diptera: Psychodidae) em cavernas do estado de Minas Gerais, Brasil (publicado no periódico Journal of Medical Entomology); 2) Substituição de espécies e diferença de abundância entre comunidades de flebotomíneos (Diptera: Psychodidae) de cavernas (será submetido no mesmo periódico). O primeiro artigo teve como objetivo testar a hipótese que prediz que o interior (zona afótica) das cavernas possuem maior riqueza e abundância de espécies de flebotomíneos durante o período noturno do que durante o período diurno e o segundo capítulo foi testar se existe alta diversidade beta entre cavernas e seus entornos, e se essa alta diversidade beta realmente existe, ela é mais influenciada pela substituição de espécies do que por diferença de abundância.

Referências

Alves, V. R., R. A. Freitas, and T. V. Barrett. 2008. *Lutzomyia maruaga* (Diptera: Psychodidae), a new bat-cave sand fly from Amazonas, Brazil. Mem. Inst. Oswaldo Cruz. 103: 251-253.

Alves, V. R., R. A. De Freitas, F. L. Santos, and T. V. Barrett. 2011. Diversity of sandflies (Psychodidae: Phlebotominae) captured in sandstone caves from Central Amazonia, Brazil. Mem. Inst. Oswaldo Cruz. 106: 353–359.

Alexander, B. and M. C. Usma. 1994. Potential source of sugar for the phlebotomine sandfly *Lutzomyia youngi* (Diptera: Psychodidae) in a Colombian coffee plantation. Ann. Trop. Med. Parasit. 88: 543-549.

Amato, V. S., H. F. De Andrade, and M. I. Duarte. 2003. Mucosal Leishmaniasis: in situ characterization of the host inflammatory response, before and after treatment. Acta Trop. 85: 39-49.

Ashford, R. W. 2000. The leishmaniases as emerging and reemerging zoonoses. Int. J. Parasitol. 30: 1269-1281.

Barral, A., D. Pedral-Sampaio, and G. Grimaldi Jr. 1991. Leishmaniasis in Bahia, Brazil: evidence that *Leishmania amazonensis* produces a wide spectrum of clinical disease. Am. J. Trop. Med. Hyg. 44: 536–546.

Carvalho, G. M. D. L., R. P. Brazil, C. C. Sanguinette, **and J.D. Andrade Filho. 2011.** Description of *evandromyia spelunca*, a new phlebotomine species of the cortelezzii complex, from a cave in Minas Gerais State, Brazil (Diptera: Psychodidae: Phlebotominae). Parasit Vectors 4 (158): 1-8.

Carvalho, G. M. L., R. P. Brazil, L. Saraiva, P. F. Quaresma, H. A. Botelho, M. C. N. F. Da Ramos, A. P. L. A. Zenóbio, P. C. L. S. Meira, C. C. Sanguinette, and J. D. Andrade Filho. 2012. Hourly Activity and Natural Infection of Sandflies (Diptera: Psychodidae) Captured from the Aphotic Zone of a Cave, Minas Gerais State, Brazil. Plos One 7 (12): 1-6.

Carvalho, G. M. D. L., R. P. Brazil, M. C. D. N. F. Ramos, P. C. L. Serra and Meira, A. P. L. D. A. Zenóbio, H. A. Botelho, C. C. Sanguinette, L. Saraiva, and J. D. Andrade Filho. 2013. Ecological Aspects of Phlebotomine Sandflies (Diptera: Psychodidae) from a Cave of the Speleological Province of Bambuí, Brazil. Plos One 8 (10): 1–10.

Campos, A. M. , R. Matavelli, C. L.C. D. Santos, L. S. Moraes, and J. M. M. Rebêlo. 2013. Ecology of phlebotomines (Diptera: Psychodidae) in a transitional area between the amazon and the cerrado in the state of maranhão, Brazil. J. Med. Entomol. 50: 52–58.

CECAV. 2016. Centro Nacional de Pesquisa e Conservação de Cavernas. Base de Dados de Cavidades Naturais. (http://www.icmbio.gov.br/cecav/downloads/mapas.html) (acessado 10 Abril 2016).

Deane, L.M., e M.P. Deane. 1954. Encontro de *Leishmania* nas vísceras e na pele de uma raposa em zona endêmica de calazar, nos arredores de Sobral. O Hospital. 45: 419-421.

Deane, L. M., e M. P. Deane. 1957. Observações sobre abrigos e criadouros de flebótomos no noroeste do Estado do Ceará. Rev. Bras. Malar. Doen. Trop. 9: 225-246.

Desjeux, P. 2004. Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect. Dis. 27: 305–318.

Do Carmo, F. F., F. F. Do Carmo, A. A. R. Salgado, and C. M. Jacobi. 2011. Novo sítio espeleológico em sistemas ferruginosos, no Vale do Rio Peixe Bravo, norte de Minas Gerais , Brasil. Espeleo-tema. 22.

Fernandes, G. W., N. P. U. Barbosa, D. Negreiros, and A. P. Paglia. 2014. Challenges for the conservation of vanishing megadiverse rupestrian grasslands. Nat. Conserv. 12(2): 162–5.

Ferreira, R. L., and L. C. Horta. 2001. Natural and human impacts on invertebrate communities in Brazilian caves. Braz. J. Biol. 61: 7–17.

Ferreira, R. L., L. Ferreira, D. O. Bernardi, e M. S. Silva. 2009. Caracterização dos ecossistemas das Grutas Aroê Jari , Kiogo Brado e Lago Azul (Chapada dos Guimarães, MT): Subsídios para o turismo nestas cavidades. Rev. Biol. e Ciências da Terra. 9: 41–58.

Forattini, O. P. 1960. Novas observações sobre a biologia de flebótomos em condições naturais (Diptera:Psychodidae). Arch. Hyg. Saúde Publ. 25: 209-215.

Forattini, O. P. 1973. Entomologia médica. São Paulo: Edgard Blucher. v. 4.

Fuenzalida, A. D., M. G. Quintana, O. D. Salomón, and M. S. L. Grosso. 2011. Hourly activity of Lutzomyia neivai in the endemic zone of cutaneous leishmaniasis in Tucumán, Argentina: preliminary results. Mem. Inst. Oswaldo Cruz 106: 635–638.

Galati, E. A. B., J. D. Andrade Filho, A. C. L. Silva, and A. L. Falcão. 2003a. Description of a new genus and new species of New World Phlebotominae (Diptera, Psychodidae). Rev. Bras. Entomol. 47: 63-70.

Galati, E. A. B., A. M. Marassá, and R. M. G. Andrade. 2003b. *Micropygomyia* (*Sauromyia*) *petari*, a new species of Phlebotominae (Diptera: Psychodidae) from Vale do Ribeira, São Paulo state, Brazil. Rev. Bras. Entomol. 47: 455-459.

Galati, E. A. B., V. L. B. Nunes, P. C. Boggiani, M. E. C. Dorval, G. Cristaldo, H. C. da Rocha, E. T. Oshiro, R. M. Gonçalves-de-Andrade, and G. Naufel. 2003c. Phlebotomines (Diptera: Psychodidae) in caves of the Serra da Bodoquena, Mato Grosso do Sul State, Brazil. Rev. Bras. Entomol. 47: 283–296.

Galati, E. A. B., V. L. B. Nunes, P. C. Boggiani, M. E. C. Dorval, G. Cristaldo, H. C. da Rocha, E. T. Oshiro, R. M. Gonçalves-de-Andrade, and G. Naufel. 2006. Phlebotomines (Diptera: Psychodidae) in forested areas of the Serra da Bodoquena, state of Mato Grosso do Sul, Brazil. Mem. Inst. Oswaldo Cruz. 101: 175–193.

Galati, E. A. B., A. M. Marassá, R. M. G Andrade, E. F. M. Bueno, B. R. Paiva, and R. S. Malafronte. 2010. *Nyssomyia intermedia* (Lutz & Neiva) and

Nyssomyia neivai (Pinto) (Diptera, Psychodidae, Phlebotominae) in a sympatric area: seasonal and nocturnal hourly rhythm in black and white modified Shannon traps. Rev. Bras. Entomol. 54: 677–686.

Gontijo, C. M. F., e M. N. Melo. 2004. Leishmaniose visceral no Brasil: quadro atual, desafios e perspectivas. Rev. Bras. Entomol. 7: 338-349.

Grimaldi Jr, G., and R. B. Tesh. 1993. Leishmaniases of the New World: Current concepts and implications for future research. Clin. Microbiol. Rev. 6: 230-250.

Guernaoui, S., S. Boussaa, B. Pesson, and A. Boumezzough. 2006. Nocturnal activity of phlebotomine sand flies (Diptera:Psychodidae) in a cutaneous leishmaniasis focus in Chichaoua, Morocco. Parasitol. Res. 98: 184-8.

Holsinger, R., and D. C. Culver. 1988. The Invertebrate Cave Fauna of Virginia and a Part of Eastern Tennessee: Zoogeography and Ecology. *Brimleyana* 14: 1–162.

IEF. 2016. Instituto Estadual de Florestas. (http://www.ief.mg.gov.br/). (accessed 10 July 2016).

Jacobi, C. M., and F. F. Do Carmo. 2008. Diversidade dos campos rupestres ferruginosos no Quadrilátero Ferrífero, MG. Megadiversidade. 4: 25–33.

Lainson, R., J. J. Shaw, L. Ryan, and F. T. Silveira. 1985. Leishmaniasis in Brazil XXI. Visceral leishmaniasis in the Amazon Region and further observations on the role of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) as the vector. Trans. R. Soc. Trop. Med. Hyg. 79: 223-226.

Lainson, R., and J. J. Shaw. 1998. New World leishmaniasis: The Neotropical *Leishmania* species. *In* L. Collier, A. Baeows, M. Sussman (ed.), Microbiology and Microbial Infections. 5: 241-266.

Matavelli, R., A. M. Campos, R. N. Feio, and R. L. Ferreira. 2015. Occurrence of anurans in brazilian caves. Acta Carsol. 44: 107–120.

Nadim, A., and Faghih M. 1968. The epidemiology of cutaneous Leishmaniasis in the Isfahan province of Iran. Trans. R. Soc. Trop. Med. Hyg. 61: 534-549.

Polseela, R., A. Vitta, S. Nateeworanart, and C. Apiwathnasorn. 2011. Distribution of cave dwelling phlebotomine sand flies and their nocturnal and diurnal activity in Phitsanulok Province, Thailand. Se. Asian J. Trop. Med. 42: 1395-1404.

Poulson, T. L., and W. B. White. 1969. The Cave Environment. Science 165: 971–981.

Rangel, E. F. and R. Lainson. 2009. Proven and putative vectors of American cutaneous leishmaniasis in Brazil: aspects of their biology and vectorial competence. Mem. Inst. Oswaldo Cruz. 104: 937-954.

Ready, P. 2013. Biology of Phlebotomine Sand Flies as Vectors of Disease Agents. Annual Rev. Entomol. 58: 227-250.

Rotureau, B., C. Ravel, P. Couppié, F. Pratlong, M. Nacher, J. P. Dedet, and B. Carme. 2006. Use of PCR-restriction fragment length polymorphism analysis to identify the main newworld *Leishmania* species and analyze their taxonomic properties and polymorphism by application of the assay to clinical samples. J. Clin. Microbiol. 44: 459–467.

SBE. 2016. Sociedade Brasileira De Espeleologia. Cadastro Nacional de Cavernas do Brasil (CNC). Campinas. (http://www.cavernas.org.br/cnc_form.asp) (acessado 25 de julho de 2016).

Saraiva, L., R. A Silva, N. R. J. Marteleto, P. A. A Sampaio, F. D. Rêgo, M. R. A. C. Vianna, C. M. F. Gontijo, and J. D. Andrade Filho. 2015. Survey of Sand

Flies (Diptera: Psychodidae) in an Environmentally Protected Area in Brazil. Plos One 10: 1–5.

Shaw, J. J. 2003. New world leishmaniasis: the ecology of leishmaniasis and the diversity of leishmanial species in Central and South America. p. 11-31. *In* J. Farrel (ed). World Class Parasites: *Leishmania*. Boston, Dordrecht, London: Kluwer Academic Publishers.

Sherlock, I. A., e V. A. Sherlock. 1972. Métodos práticos para criação de flebotomíneos em laboratório. Rev. Bras. Biol. 32: 209–217.

Sherlock, I. A. 2003. A importância dos flebotomíneos. In: E. F. Rangel, R. Lainson. Flebotomíneos do Brasil. Rio de Janeiro: Fiocruz. 15-21.

Sonter, L. J., D. J. Barrett, B. S. Soares-filho, and C. J. Moran. 2014. Global demand for steel drives extensive land-use change in Brazil's Iron Quadrangle. Glob Environ Chang. 26: 63–72.

Teodoro, U., D. Albertonb, J. B. Kühl, E. S. Santos, D. R. Santos, A. R. Santos, O. Oliveira, and T. G. Verzignassi. 2003. Ecology of *Lutzomyia* (*Nyssomyia*) *whitmani* in an urban area in Brazil. Rev. Saúde Públ. 37: 651-656.

Trajano, E., and P. Gnaspini-Netto. 1991. Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos taxons. Rev. Bras. Zool. 7: 383–407.

Young, D. G, and M. A. Duncan. 1994. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Memoirs of the American Entomological Institute. 54: 881 pp.

WHO. 2010. World Health Organization Leishmaniasis: background information. Disponível em: www.who.int/leishmaniasis/en/ 2010.

WHO. 1990. World Health Organization Leishmaniasis: The control of leishmaniases: report of an expert committee. WHO Technical Report Series. 793: 50-55.

Chapter 1- Photoperiod differences in sand fly (Diptera: Psychodidae) species richness and abundance in caves in Minas Gerais state, Brazil

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Population and Community Ecology

Photoperiod Differences in Sand Fly (Diptera: Psychodidae) Species Richness and Abundance in Caves in Minas Gerais State, Brazil

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Abstract

Caves are unique habitats that are inhabited by a diverse and singular biota. Among these inhabitants are sand flies, which are of great epidemiological interest in the Neotropical region because they are vectors of *Leishmania*. The period of activity of these insects is usually crepuscular and nocturnal, but there are reports of diurnal activity of sand flies in caves. Thus, the aim of this study was to evaluate the periodicity of daily activity of sand flies in cave environments in the municipality of Pains, Minas Gerais. Sand flies were collected with light traps, which were operated for 5 consecutive days in the rainy season and in the dry season. Samples were collected every 12 h and separated between photophase and scotophase periods. In total, 1,777 sand flies of 23 species were collected. The most abundant species was *Lutzomyia renei* (Martins, Falcão, and Silva) (44%), followed by *Lutzomyia longipalpis* (Lutz and Neiva) (15%), *Evandromyia edwardsi* (Mangabeira) (11%), and *Micropygomyia quinquefer* (Costa Lima) (6%). The richness and abundance of total sand flies and the abundance of male and female sand flies in the aphotic zone of the caves did not differ between the photophase and scotophase, but differed between photoperiods at the entrance and at sites surrounding the caves. From our study of the daily activity of these insects in this ecotope, it will be possible to know which period of the day is of greatest risk of exposure of vertebrates who visit or live in these environments, including the human population.

Key words: aphotic zone, period of activity, photophase, scotophase, vector insect

Caves are stable environments, with little variation in the temperature and humidity of the interior microclimate, which is important for their ecosystems (Poulson and White 1969). In addition, cave environments lack light in areas distant from the entrance and present oligotrophic conditions (Trajano and Gnaspini-Netto 1991).

Among the animals that frequent cave environments are sand flies (Galati et al. 2003a, 2003b, 2003c; Alves et al. 2008; Carvalho et al. 2011, 2012, 2013; Polseela et al. 2011; De Oca-Aguilar et al. 2013; Saraiva et al. 2015). These insects are natural vectors of several microorganisms, especially protozoa of the genus *Leishmania* Ross (Forattini 1973, Young and Duncan 1994). Generally, sand flies are crepuscular and/or nocturnal, resting in natural shelters during the day, although some species may exhibit different patterns of daily activity under certain conditions (Teodoro et al. 2003, Young and Duncan 1994, Sherlock 2003).

In caves, some species of sand flies are active during the day (Carvalho et al. 2012). Some studies have shown that the period of activity of sand flies in the aphotic zones of caves, as well as in anthropic environments, is greater in the scotophase than in the photophase, indicating that they exhibit a nocturnal circadian rhythm (Guernaoui et al. 2006, Galati et al. 2010, Fuenzalida et al. 2011, Polseela et al. 2011). However, a study conducted in a cave in the municipality of Manaus, Amazonas, found different results, with the daily activity of a species of sand fly in the interior of the cave not differing between nocturnal and diurnal periods (Alves et al. 2011). Sand flies in caves may modulate their daily activity pattern in response to bats occupancy. Bats use caves as a shelter during the day and leave during the night to search for food (Lampo et al. 2000) and may be a source of blood for sand flies.

Little is known of the behavior of sand flies in the caves. Knowledge of the daily activity of these insects in this ecotope will describe the period of the day when there is the greatest exposure of vertebrates who visit or live in these environments to blood-feeding sand flies, including the human population. Although the studied caves are not tourist attractions, the results found in our study may be extrapolated to tourist caves where there is a large flow of people and thus greater contact with these insects, which may be important for understanding the risk of transmission of *Leishmania*.

Therefore, the aim of this study was to determine the period of daily activity of sand flies in five cave environments in the municipality of Pains, state of Minas Gerais. We tested the hypothesis that cave interiors (aphotic zone) possess greater species richness and abundance of sand flies during the nocturnal period compared with the diurnal period. This hypothesis was based on the fact that the sand flies are nocturnal, and despite the constant darkness in the aphotic zone, they are expected to respond to a circadian cycle with a greater number of individuals and species searching for food during the nocturnal period.

Materials and Methods

Study Area

The municipality of Pains forms part of a complex that possesses a large number of natural caves (CECAV 2015). The caves used in this study are localized in limestone outcroppings, contain water bodies, and receive water from outside the cave from dripping, which can create temporary ponds and even cause flooding during the rainy season. This region has the phytogeographic features of "Mata de Pains" or Deciduous *Seasonal* Forest and Cerrado (Ururahy et al. 1983). The area suffers from anthropic disturbance from mining, calcining, and agriculture.

The study took place in five cave environments and their surroundings in the municipality of Pains, state of Minas Gerais, Brazil: Gruta Santuário (20° 25′14″ S and 45° 46′26″ W), which is located at an altitude of 708 m above sea level (a.s.l.); Gruta Brega (20° 25′05″ S and 45° 46′19″ W) – 712 m a.s.l.; Gruta Camilo (20° 20′46″ S and 45° 40′58″ W) – 702 m a.s.l.; Gruta Isaias (20° 22′05″ S and 45° 39′28″ W) – 732 m a.s.l.; and Gruta Morrinho das Pedras (20° 21′25″ S and 45° 39′53″ W) – 678 m a.s.l. Gruta Camilo, Gruta Isaias, and Gruta Morrinho das Pedras have an aphotic zone about 20 m distance interior from the entrance, whereas in Gruta Santuário and Gruta Brega, the aphotic zone is at 40 and 100 m, respectively. All the caves were named by Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV). In these caves, we observed vertebrates including lizards, snakes, rodents, and bats (Fig. 1).

Collection of Sand Flies

The collection of sand flies was performed under the license N°45636-1 of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis.

Sand flies were collected at each site using paired model HP automatic light traps (Pugedo et al. 2005), with one trap set from 0600 to 1800 h and a second trap set from 1800 to 0600 h. Traps were operated uninterruptedly for two consecutive 120-h periods over 5 d of sampling in the rainy season (November 2014) and in the dry season (April 2015), for a total of 240 h sampling effort for the two traps.

At each cave and its surroundings, pairs of traps were set at three sampling points: one located at the cave entrance (the photic zone), one in the interior of the cave (aphotic zone), and one in the surrounding area (about 10 m from the cave entrance), for a total of 15 trapping points at the five caves and their surroundings in each season. The traps were installed approximately 1 m above the ground in all cave environments.

After sampling, the traps were removed, the sand flies killed in glycerinated alcohol, sorted, and sexed. Males were then placed in test tubes containing 70% alcohol and females were placed in test tubes containing 6% DMSO.

Sand Fly Identification

Male specimens were mounted in Canada Balsam and identified following the classification of Galati (2003). For female identification, the head and the last three segments of the abdomen were separated and mounted in Berlese medium, with the head positioned ventrally. All specimens were deposited in the Coleção de Flebotomíneos of the Centro de Pesquisas René Rachou/Fiocruz (FIOCRUZ/COLFLEB).

Due to the morphological similarity of the genitalia of female sand flies of the genus *Brumptomyia* França and Parrot, and the occurrence of males of three species of this genus in the studied cave environments, species identification of females of this genus was difficult. Thus, males of *Brumptomyia* were identified to species and females only to genus (i.e., *Brumptomyia* sp.).

Statistical Analysis

The effect of sampling period (diurnal and nocturnal) on the sex, richness, and abundance of sand flies collected in the interior, entrance, and surroundings of the caves was determined using a generalized linear mixed model (GLMM; Zuur et al. 2009). In these models, the sums of the richness and abundance of sand flies and the sums of the abundance of females and of males of sand flies for the 5 consecutive days of sampling of the interior, entrance, and surrounding of each cave in each season were used as response variables, while the sampling period (diurnal and nocturnal) was used as an explanatory variable and the caves and year seasons (repeated measures) as a random variable. All models were performed using the software R 3.2.4 (R Development Core Team 2016), followed by residual analysis to choose the best data distribution, which for our data was the negative binomial distribution.

Results

In total, 1,777 phlebotomine sand flies were collected, including 489 (27.5%) males and 1,288 (72.5%) females, of 23 species belonging to 11 genera (Table 1). The most abundant species was *Lutzomyia renei* (Martins, Falcão and Silva) (44%), followed by *Lutzomyia longipalpis* (Lutz and Neiva) (15%), *Evandromyia edwardsi* (Mangabeira) (11%), and *Micropygomyia quinquefer* (Costa Lima) (6%). All other species together amounted to 24% of the total (Table 1).

Cave entrances yielded 838 (47%) individuals, with the most abundant species being *Lu. renei* (39%) and *Lu. longipalpis* (26%), whereas 635 (36%) individuals were collected from the cave interiors, with *Lu. renei* (68%) also being the most abundant species. In the cave surroundings, 304 (17%) individuals were collected, with *Mi. quinquefer* (18%) being the most abundant. All species recorded in the cave interiors and surroundings were also found at the

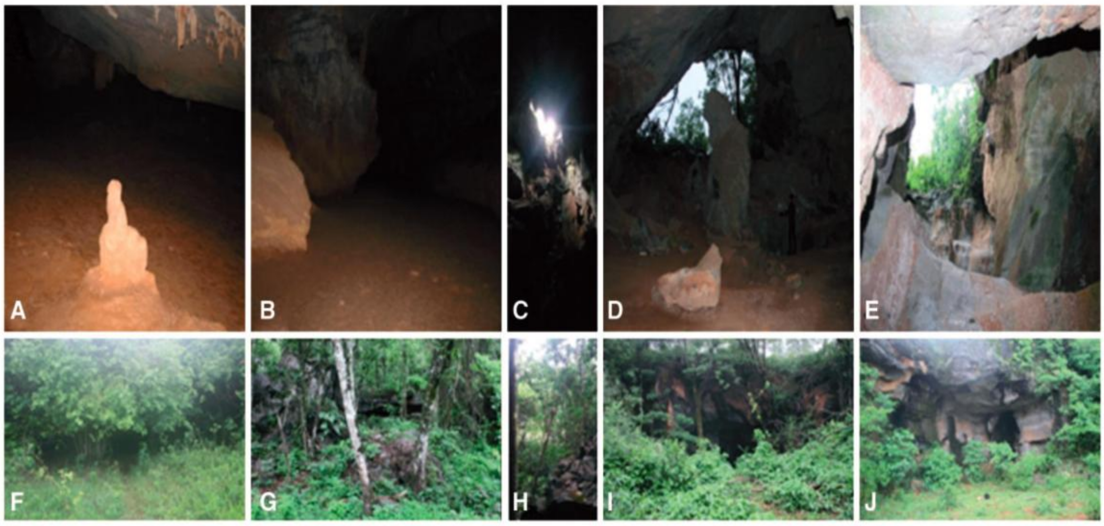

Fig. 1. The study caves and their surroundings in the municipality of Pains, Minas Gerais, Brazil. A and F, Gruta Santuário and surroundings; B and G, Gruta Brega and surroundings; C and H, Gruta Camilo and surroundings; D and I, Gruta Isaias and surroundings; and E and J, Gruta Morrinho das Pedras and surroundings.

Table 1. Phlebotomines collected from caves and their surroundings in the municipality of Pains-MG, in November 2014 and April 2015

Cave environments/Periods

Species	Entrance						Total	Interior						Total	Surroundings						Total	Total
	Diurnal		Total	Nocturnal		Total		Diurnal		Total	Nocturnal		Total		Diurnal		Total	Nocturnal		Total		
	♀	♂		♀	♂			♀	♂		♀	♂			♀	♂		♀	♂			
Brumptomyia avellari	–	1	1	–	1	1	2	–	1	1	–	3	3	4	–	–	–	–	16	16	16	22
Brumptomyia brumpti	–	–	–	–	2	2	2	–	–	–	–	3	3	3	–	–	–	–	14	14	14	19
Brumptomyia nitzulescui	–	–	–	–	7	7	7	–	–	–	–	1	1	1	–	–	–	–	1	1	1	9
Brumptomyia sp.	2	–	2	7	–	7	9	1	–	1	2	–	2	3	–	–	–	39	–	39	39	51
Evandromyia bacula	–	–	–	1	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Evandromyia cortelezzii	4	–	4	15	5	20	24	13	2	15	8	2	10	25	1	2	3	16	7	23	26	75
Evandromyia edwardsi	2	–	2	43	10	53	55	37		37	62	18	80	117	2	–	2	25	5	30	32	204
Evandromyia lenti	–	–	–	2	1	3	3	–	–	–	–	–	–	–	–	–	–	9	1	10	10	13
Evandromyia termitophila	–	–	–	1	–	1	1	–	1	1	–	–	–	1	–	–	–	2	1	3	3	5
Expapillata firmatoi	–	–	–	3	6	9	9	–	–	–	1	1	2	2	–	1	1	1	–	1	2	13
Lutzomyia longipalpis	2	3	5	49	168	217	222	2	13	15	4	6	10	25	–	–	–	9	21	30	30	277
Lutzomyia renei	159	8	167	155	8	163	330	250	19	269	132	17	149	418	3	–	3	27	5	32	35	783
Micropygomyia quinquefer	1	9	10	10	17	27	37	7	1	8	3	3	6	14	7	3	10	18	28	46	56	107
Migonemyia migonei	3	–	3	26	9	35	38	2	–	2	2	1	3	5	2	–	2	1	2	3	5	48
Nyssomyia neivai	–	–	–	1	–	1	1	–	–	–	–	1	1	1	–	–	–	3	2	5	5	7
Nyssomyia whitmani	–	–	–	1	–	1	1	–	–	–	–	–	–	–	–	–	–	4	1	5	5	6
Pintomyia christenseni	–	–	–	1	–	1	1	–	–	–	–	–	–	–	–	–	–	1	–	1	1	2
Pintomyia fischeri	–	–	–	–	1	1	1	–	–	–	–	–	–	–	–	–	–		1	1	1	2
Pintomyia monticola	1	–	1	6	–	6	7	–	–	–	–	–	–	–	–	–	–	6	1	7	7	14
Pintomyia pessoai	1	–	1	7	6	13	14	–	–	–	–	–	–	–	1	–	1	5	–	5	6	20
Psathyromyia aragaoi	–	–	–	–	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
Psathyromyia lutziana	–	–	–	2	1	3	3	3	2	5	–	1	1	6	–	–	–	–	–	–	–	9
Sciopemyia microps	5	–	5	5	–	5	10	2	–	2	–	–	–	2	1	–	1	–	–	–	1	13
Sciopemyia sordellii	6	5	11	38	10	48	59	4	–	4	4	–	4	8	2	–	2	5	2	7	9	76
Total specimens per sex	186	26	**212**	373	253	**626**	**838**	321	39	**360**	218	57	**275**	**635**	19	6	**25**	171	108	**279**	**304**	**1,777**
Total species	11	5	**12**	19	16	**24**	**24**	10	7	**12**	9	12	**14**	**16**	8	3	**9**	16	16	**20**	**21**	**23**

entrance of the caves, but some species were exclusively found at the entrance.

During the diurnal period, 597 (34%) phlebotomine sand flies were collected, consisting of 360 (60%) individuals in the cave interiors, 212 (36%) in the cave entrances, and 25 (4%) in the cave surroundings. The most abundant species during the diurnal period was *Lu. renei* (73%). The nocturnal period yielded 1,180 (66%) individuals, with 275 (23%) in the cave interiors, 626 (53%) in the cave entrances, and 279 (24%) in the cave surroundings, with *Lu. renei* (29%), *Lu. longipalpis* (22%), and *Ev. edwardsi* (11%) being the most abundant species in these three cave environments.

All species recorded during the diurnal period were also found during the nocturnal period, but some species such as *Brumptomyia brumpti* (Larrousse), *Brumptomyia nitzulescui* (Costa Lima), *Evandromyia bacula* (Martins, Falcão and Silva), *Psathyromyia aragaoi* (Costa Lima), *Evandromyia lenti* (Mangabeira), *Nyssomyia whitmani* (Antunes and Coutinho), *Nyssomyia neivai* (Pinto), *Pintomyia christenseni* (Young and Duncan), and *Pintomyia fischeri* (Pinto) were exclusively collected during the nocturnal period.

At cave entrances during the diurnal period, the most abundant species was *Lu. renei* (79%), whereas during the nocturnal period, *Lu. longipalpis* (35%) was most common, followed by *Lu. renei* (26%). In cave interiors (aphotic area), *Lu. renei* was the most abundant species during both the diurnal and nocturnal periods (75 and 54%, respectively). In cave surroundings, *Mi. quinquefer* was always the most abundant species (40% diurnal and 16% nocturnal).

Comparing the richness and abundance of sand flies between diurnal and nocturnal periods of the interior, entrance, and surrounding environments of the caves, we observed that the richness and abundance in the cave interiors did not differ between diurnal and nocturnal periods ($z = 1.19$, $P = 0.23$; $z = 1.13$, $P = 0.19$, respectively), but differed between diurnal and nocturnal periods in the entrance and surrounding of the caves, where there was a greater richness and abundance of sand flies during the nocturnal period (Abundance: $z = 3.30$, $P = 0.009$ and Richness: $z = 4.42$, $P < 0.001$; Abundance: $z = 5.19$, $P < 0.001$ and Richness: $z = 5.51$, $P < 0.001$, respectively; Fig. 2).

The abundance of female and male sand flies in the cave interiors did not differ between diurnal and nocturnal periods ($z = 1.46$, $P = 0.143$; $z = 1.93$, $P = 0.054$, respectively), while for entrance and surrounding of the caves, there were significant differences between the periods, with a greater abundance of females and males during the nocturnal period (Females: $z = 3.33$, $P = 0.008$ and $z = 4.49$, $P < 0.001$; Males: $z = 3.47$, $P = 0.0005$ and $z = 4.48$, $P < 0.001$, respectively; Fig. 2).

Discussion

The original hypothesis of this study was refuted, because we did not find a significant difference in richness and abundance of sand flies between nocturnal and diurnal periods in the aphotic region of

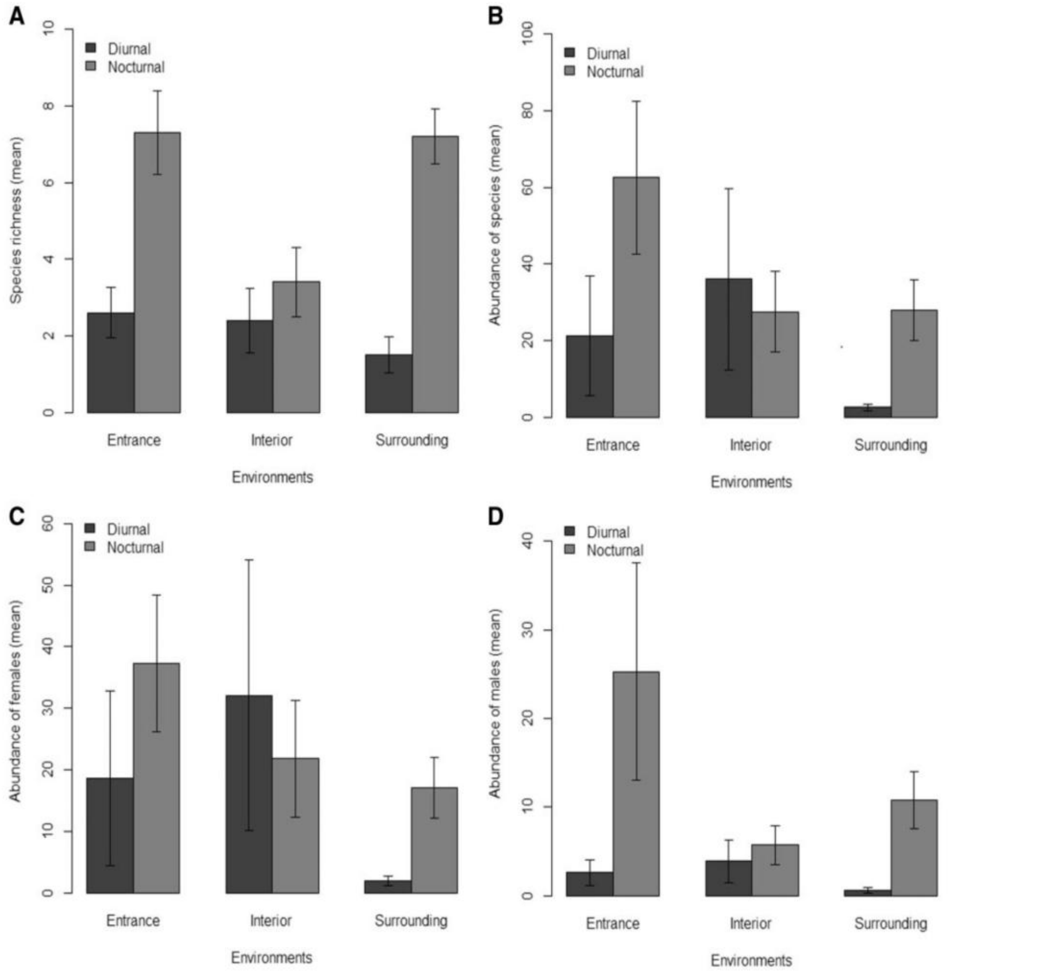

Fig. 2. Effect of sampling period (diurnal and nocturnal) on the sex, richness, and abundance of sand flies collected in the interior, entrance, and surrounding environments of the caves. A, Mean (± standard error) species richness of sand flies; B, mean (± standard error) abundance of species of sand flies; C, mean (± standard error) abundance of females of sand flies; D, mean (± standard error) abundance of males of sand flies.

the caves. This result indicated that in the cave interiors, these insects are active during both periods, which may be due to continuous resource availability. We also have not found a difference in the abundance of females and males between the photoperiods which would support that these insects are active during both periods.

Our data also suggest that in the cave interiors, entrances, and surrounding areas, sand flies exhibit differences in their activity rhythms due to the presence of sunlight that suppressed the flight activity of these insects which are mainly nocturnal.

The most abundant species in our study, *Lu. renei*, *Lu. longipalpis*, *Ev. edwardsi*, and *Mi. quinquefer*, are commonly found in cave environments (Galati et al. 2003c, 2010a; Carvalho et al. 2012, 2013). We observed that some species were collected only during the nocturnal period, some species only during the diurnal period, and some species in both periods. It is possible that these species use the caves as places for rest, protection, shelter, breeding, and feeding, as reported by Carvalho et al. (2013).

Some studies have reported similar results. Alves et al. (2011) did not find significant differences between diurnal and nocturnal periods in the number of individuals of the sand fly *Deanemyia maruaga* (Alves.Freitas & Barrett) inside a cave in the municipality of Manaus, Amazonas. Likewise, Carvalho et al. (2012) found a similar result for the distribution of total sand flies captured in the aphotic zone of a cave in the municipality of Lassance, Minas Gerais, but these authors detected peak of abundance at certain hours of both photophase and scotophase periods. Yet a study performed by Polseela et al. (2011) in caves in Thailand reported results that differed from our study, with a greater number of sand flies during the nocturnal period.

Our study also showed that all species present in the cave interiors and surroundings were also recorded at the cave entrance, which suggests that sand flies were circulating throughout these environments. A similar result was reported by Carvalho et al. (2013), who found that all the species recorded inside a cave in the municipality of Lassance, Minas Gerais, were also present in the surroundings.

One interesting and relevant finding of the present study was the high number of females captured in the caves (interior and entrance). This is significant because they feed on vertebrates and therefore can transmit *Leishmania*, the causative agent of leishmaniasis (Morrison et al. 1993, Colmenares et al. 1995, Killick-Kendrick 1999). This finding may be due to a greater opportunity for females to feed on the blood of the variety of species of vertebrates present in caves during the diurnal and nocturnal periods (Matavelli et al. 2015). Of the five caves sampled in our study, bats and rodents were captured during the nocturnal period at the entrance and interior of four of these caves (unpublished data) and probably during the diurnal period, these small mammals also used the caves as shelters. According to Gomes and Galati (1987), the predominance of females can also be explained by their attraction to both the light of traps and the availability of a blood supply near the traps.

The presence of species incriminated in *Leishmania* transmission, such as *Lu. longipalpis*, *Migonemyia migonei* (França), *Ny. neivai*, and *Ny. whitmani*, deserves special attention. The presence of these species inside and around the caves can be crucial to the sylvatic transmission cycle of *Leishmania* between reservoirs present in these environments and humans, who can become infected accidentally when visiting the caves.

Understanding the daily activity of sand flies in cave environments is important for determining the period of risk of *Leishmania* transmission between the sand flies and the vertebrate hosts who visit or live in these caves. All studied cave environments are close to human habitation, and therefore, there is the possibility of rural transmission cycle with spillover to humans in this region. The female sand flies feed on blood of many vertebrates (Brazil and Brazil 2003), including bats (Tesh et al. 1971, Christensen and Herrer 1980, Lampo et al. 2000). During the night, bats exit the caves in search of food, and many species are close to human habitations. In these habitations, the sand flies that have more nocturnal habits may become infected when they feed on these bats (De Lima et al. 2008, Savani et al. 2010, Shapiro et al. 2013) and may then transmit *Leishmania* to synanthropic animals, domestic (dog), and/or man in their next bloodmeal and, therefore, the *Leishmania* transmission cycle may be completed. The study of the habits of the sand flies, such as the present study, facilitates proper planning for human avoidance of contact with these insects in these environments.

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References Cited

Alves, V. R., R. A. Freitas, and T. V. Barrett. 2008. *Lutzomyia maruaga* (Diptera: Psychodidae), a new bat-cave sand fly from Amazonas, Brazil. Mem. Inst. Oswaldo Cruz 103: 251–253.

Alves, V. R., R. A. De Freitas, F. L. Santos, and T. V. Barrett. 2011. Diversity of sandflies (Psychodidae: Phlebotominae) captured in sandstone caves from Central Amazonia, Brazil. Mem. Inst. Oswaldo Cruz 106: 353–359.

Brazil, P. P., and B. G. Brazil. 2003. Biologia de flebotomíneos neotropicais, pp. 257–274. *In*: E. F. Rangel, and R. Lainson (eds.), Flebotomíneos do Brasil. Fiocruz, Rio de Janeiro, RG.

Carvalho, G.M.D.L., R. P. Brazil, C. C. Sanguinette, and J. D. Andrade Filho. 2011. Description of *evandromyia spelunca*, a new phlebotomine species of the cortelezzii complex, from a cave in Minas Gerais State, Brazil (Diptera: Psychodidae: Phlebotominae). Parasit Vectors 4: 1–8.

Carvalho, G.M.L., R. P. Brazil, L. Saraiva, P. F. Quaresma, H. A. Botelho, M.C.N.F. Da Ramos, A.P.L.A. Zenóbio, P.C.L.S. Meira, C. C. Sanguinette, and J. D. Andrade Filho. 2012. Hourly activity and natural infection of sandflies (Diptera: Psychodidae) captured from the aphotic zone of a cave, Minas Gerais State, Brazil. PLoS ONE 7: 1–6.

Carvalho, G.M.D.L., R. P. Brazil, M.C.D.N.F. Ramos, P.C.L. Serra e Meira, A.P.L.D.A. Zenóbio, H. A. Botelho, C. C. Sanguinette, L. Saraiva, and J. D. Andrade Filho. 2013. Ecological aspects of phlebotomine sandflies (Diptera: Psychodidae) from a cave of the speleological province of Bambuí, Brazil. PLoS ONE 8:

CECAV 2015. Centro Nacional de Pesquisa e Conservação de Cavernas. 2016. Base de Dados de Cavidades Naturais. (http://www.icmbio.gov.br/cecav/downloads/mapas.html) (accessed 10 April 2016).

Christensen, H. A., and A. Herrer. 1980. *Panamanian Lutzomyia* (Diptera: Psychodidae) host attraction profiles. J. Med. Entomol. 17: 522–528.

Colmenares, M., M. Portús, J. Botet, C. Dobaño, M. Gállego, M. Wolff, and G. Seguí. 1995. Identification of blood meals of *Phlebotomus perniciosus* (Diptera: Psychodidae) in Spain by a Competitive Enzyme Linked Immunosorbent Assay Biotin/Avidin Method. J. Med. Entomol. 32: 229–233.

De Lima, H., N. Rodríguez, M. A. Barrios, Á. Ávila, I. Cañizales, and S. Gutiérrez. 2008. Isolation and molecular identification of *Leishmania chagasi* from a bat (*Carollia perspicillata*) in northeastern Venezuela. Mem. Inst. Oswaldo Cruz 103: 412–414.

De Oca-Aguilar, A.C.M., D. Moo-Llanes, and E. A. Rebollar-Téllez. 2013. Sand Fly Species from a Karstic Cave in the Peninsula of Yucatan. Mexico. Entomol. News 123: 191–200.

Forattini, O. P. 1973. Entomologia Médica Phlebotominae. Leishmanioses e Bartoneloses. Ltda EB, São Paulo, SP.

Fuenzalida, A. D., M. G. Quintana, O. D. Salomón, and M.S.L. Grosso. 2011. Hourly activity of Lutzomyia neivai in the endemic zone of cutaneous leishmaniasis in Tucumán, Argentina: preliminary results. Mem. Inst. Oswaldo Cruz 106: 635–638.

Galati, E.A.B. 2003. Morfologia e taxonomia. Classificacão de Phlebotominae, pp. 23–51. *In* E. F. Rangel and R. Lainson (org.), Flebotomíneos do Brasil, 1st ed. Fiocruz, Rio de Janeiro.

Galati, E.A.B., J. D. Andrade Filho, A.C.L. Silva, and A. L. Falcão. 2003a. Description of a new genus and new species of New World Phlebotominae (Diptera, Psychodidae). Rev. Bras. Entomol. 47: 63–70.

Galati, E.A.B., A. M. Marassá, and R.M.G. Andrade. 2003b. *Micropygomyia* (*Sauromyia*) *petari*, a new species of Phlebotominae (Diptera: Psychodidae) from Vale do Ribeira, São Paulo state, Brazil. Rev. Bras. Entomol. 47: 455–459.

Galati, E.A.B., V.L.B. Nunes, P. C. Boggiani, M.E.C. Dorval, G. Cristaldo, H. C. da Rocha, E. T. Oshiro, R. M. Gonçalves-de-Andrade, and G. Naufel. 2003c. Phlebotomines (Diptera: Psychodidae) in caves of the Serra da Bodoquena, Mato Grosso do Sul State. Brazil. Rev. Bras. Entomol. 47: 283–296.

Galati, E.A.B., A. M. Marassá, R.M.G. Andrade, E.F.M. Bueno, B. R. Paiva, and R. S. Malafronte. 2010. *Nyssomyia intermedia* (Lutz & Neiva) and *Nyssomyia neivai* (Pinto) (Diptera, Psychodidae, Phlebotominae) in a sympatric area: seasonal and nocturnal hourly rhythm in black and white modified Shannon traps. Rev. Bras. Entomol. 54: 677–686.

Gomes, A. C., and E.A.B. Galati. 1987. Aspectos ecológicos da leishmaniose tegumentar americana. 5. Estratificação da atividade espacial e estacional de Phlebotominae (Diptera, Psychodidae) em áreas de cultura agrícola da região do Vale do Ribeira, Estado de São Paulo, Brasil. Mem. Inst. Oswaldo Cruz 82: 467–473.

Guernaoui, S., S. Boussaa, B. Pesson, and A. Boumezzough. 2006. Nocturnal activity of phlebotomine sand flies (Diptera: Psychodidae) in a cutaneous leishmaniasis focus in Chichaoua, Morocco. Parasitol. Res. 98: 184–188.

Killick-Kendrick, R. 1999. The biology and control of phlebotominae sand flies. Clinic. Dermatol. 17: 279–289.

Lampo, M., M. D. Feliciangeli, L. M. Márquez, and C. Bastidas. 2000. A possible role of bats as a blood source for the *Leishmania* vector *Lutzomyia longipalpis* (Diptera: Psychodidae). Am. J. Trop. Med. Hyg. 62: 718–719.

Matavelli, R., A. M. Campos, R. N. Feio, and R. L. Ferreira. 2015. Occurrence of anurans in Brazilian caves. Acta Carsologica 44: 107–120.

Morrison, A. C., C. Ferro, and R. Tesh. 1993. Host preference of sand fly *Lutzomyia longipalpis* at an endemic focus of American visceral leishmaniasis in Colombia. Am. J. Trop. Med. Hyg. 49: 68–75.

Polseela, R., A. Vitta, S. Nateeworanart, and C. Apiwathnasorn. 2011. Distribution of cave dwelling phlebotomine sand flies and their nocturnal and diurnal activity in Phitsanulok Province, Thailand. Se. Asian J. Trop. Med. 42: 1395–1404.

Poulson, T. L., and W. B. White. 1969. The cave environment. Science 165: 971–981.

Pugedo, H., R. A. Barata, J. C. França-Silva, J. C. Silva, and E. S. Dias. 2005. HP: Um modelo aprimorado de armadilha luminosa de sucção para a captura de pequenos insetos. Rev. Soc. Bras. Med. Trop. 38: 70–72.

R Development Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org)

Saraiva, L., R. A. Silva, N.R.J. Marteleto, P.A.A. Sampaio, F. D. Rêgo, M.R.A.C. Vianna, C.M.F. Gontijo, and J. D. Andrade Filho. 2015. Survey of sand flies (Diptera: Psychodidae) in an environmentally protected area in Brazil. PLoS ONE 10: 1–5.

Savani, E.S.M.M., M. F. De Almeida, M.C.G. de, O. Camargo, S.R.N. D'Auria, M.M.S. Silva, M. L. De Oliveira, and D. Sacramento. 2010. Detection of *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Leishmania*) *infantum chagasi* in Brazilian bats. Vet. Parasitol. 168: 5–10.

Shapiro, J. T., M. S. da, C. Lima Junior, M.E.C. Dorval, A. de, O. França, M. de, F. C. Matos, and M. O. Bordignon. 2013. First record of *Leishmania braziliensis* presence detected in bats, Mato Grosso do Sul, southwest Brazil. Acta Trop. 128: 171–174.

Sherlock, I. A. 2003. Importância médico veterinária, pp. 15–22. *In* E. F. Rangel, and R. Lainson (eds.), Flebotomíneos do Brasil. Fiocruz, Rio de Janeiro, RG.

Teodoro, U., D. Albertonb, J. B. Kühl, E. S. Santos, D. R. Santos, A. R. Santos, O. Oliveira, and T. G. Verzignassi. 2003. Ecology of *Lutzomyia* (*Nyssomyia*) *whitmani* in an urban area in Brazil. Rev. Saúde Públ. 37: 651–656.

Tesh, R. B., B. N. Chaniotis, M. D. Aronson, and K. M. Johnson. 1971. Natural host preferences of Panamanian phlebotomine sandflies as determined by precipitin test. Am. J. Trop. Med. Hyg. 20: 1–5.

Trajano, E., and P. Gnaspini-Netto. 1991. Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos taxons. Rev. Bras. Zool. 7: 383–407.

Ururahy, J.C.C., J.E.R. Collares, M. M. Santos, and R.A.A. Barreto. 1983. Folhas SF.23/24 Rio de Janeiro/Vitória, geologia, geomorfologia, pedologia, vegetação e uso potencial da terra. Vegetação. *In* Projeto RADAMBRASIL: As regiões fitoecológicas, sua natureza e seus recursos econômicos. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, RG.

Young, D. G., and M. A. Duncan. 1994. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Mem. Am. Entomol. Inst. 54: 1–881.

Zuur, A., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology. Statistics for Biology and Health. Springer, New York.

Chapter 2- Species replacement and abundance difference among phlebotomine sand fly species (Diptera: Psychodidae) of caves

Species replacement and abundance difference among phlebotomine sand fly species (Diptera: Psychodidae) of caves

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ABSTRACT Caves, though seemingly inhospitable due to the lack of light in areas furthest from the cave entrance and the low availability and variety of resources, can harbor many species of vertebrates and invertebrates, including phlebotomine sand flies, the vectors of species of parasites of the genus *Leishmania* that cause leishmaniasis. Thus, caves represent an important setting for testing hypotheses about the patterns of sand fly distribution. Here, we tested the hypothesis that there is high beta diversity between caves and their surroundings, and that this diversity, if it indeed exists, is driven more by species replacement (individuals of different species are replaced between sites) than by abundance difference (difference in the total abundances of the species between sites). The collections of sand flies were performed with automatic light traps in cave environments and their surroundings at three locations in southeastern Brazil in the state of Minas Gerais. We partitioned beta diversity into species replacement and abundance difference between cave environments and their respective surroundings to infer biological mechanisms from the patterns of distribution of sand flies between these environments. A total of 722 phlebotomine sand flies were collected with the most abundant species being *Evandromyia tupynambai* (28%), *Evandromyia edwardsi* (19%), *Brumptomyia troglodytes* (14%), and *Psychodopygus lloydi* (11%). The beta diversity between each cave and its surroundings was high, and determined mostly by species replacement in caves that had an aphotic zone and was mostly determined by abundance difference in cave that did not have an aphotic zone. Therefore, our results suggest that both caves and their surroundings are important for the maintenance of sand fly communities, and that the peculiar characteristics of caves have presumably led to differences in the sand fly communities found within and surrounding caves.

KEY WORDS: beta diversity, caves, Quadrilátero Ferrífero, vector insects.

Phlebotomine sand flies are insects of the family Psychodidae, subfamily Phlebotominae (Diptera). They are the invertebrate hosts for protozoan parasites of the genus *Leishmania*, which cause leishmaniases in humans and other mammals (Forattini 1973). These insects can be found in urban, rural and wild environments, including caves (Galati et al. 2003a, 2003b, 2003c, 2006; Alves et al. 2008, 2011; Carvalho et al. 2012, Campos et al. 2013).

Cave environments, though seemingly inhospitable due to the lack of light in areas furthest from the cave entrance and the low availability and variety of resources (Trajano and Gnaspini-Netto 1991, Prous et al. 2015), can harbor many species of vertebrates and invertebrates, including phlebotomine sand flies (Ferreira and Horta 2001, Galati et al. 2003a, 2003b, 2003c; Alves et al. 2008, Ferreira et a. 2009, Carvalho et al. 2011, 2012, 2013, Matavelli et al. 2015, Saraiva et al. 2015).

Studies conducted inside caves have produced interesting results, such as finding that the diversity of sand flies inside caves is usually greater than or equal to that in their surrounding environments (Galati et al. 2003a, b, c, 2006, 2010; Carvalho et al. 2013). Additionally, the species composition of sand fly communities of caves differ from that of their surrounding areas with some species being restricted to the caves, other species restricted to surroundings and yet others common to both environments (Galati et al 2003c, 2006, 2010; Alves et al. 2008, Carvalho et al. 2013, Campos et al. 2016). Campos et al. (2016) have also shown that sand flies exhibit differences in their activity rhythms among the cave interior, entrance and surrounding areas due to differences in the presence of sunlight, which suppresses flight activity of these mainly nocturnal insects. However, despite all these investigations, it is still not clear that we should expect high beta diversity between caves and their surroundings, even though it is known that there are different species outside and inside of caves.

Dissimilarity measures have been widely used for evaluating the distribution of different biotas and they have a long-standing history (Jacard 1912, Simpson 1943, Sørensen 1948). Recently, Podani and Schmera (2011), Carvalho et al. (2012, 2013) and Podani et al. (2013) defined beta diversity as a measure of dissimilarity between communities that is determined by two phenomena: species replacement and richness or abundance difference.

Species replacement refers to the well-known fact that species are replaced by others, or to the fact that the individuals that exceed the number of individuals of these species in other site are replaced, in this site, by the same number of individuals of different species as a consequence of environmental filtering, competition and historical events (Qian et al. 2005, Leprieur et al. 2011, Legendre 2014). Richness or abundance difference refers to the fact that one community may include a larger number of species than another, or that communities may differ mostly in the total abundance of the species present as a consequence of the diversity of niches available and other ecological processes such as physical barriers (Legendre 2014). Thus, the use of dissimilarity indexes to measure beta diversity caused by species replacement and richness or abundance difference are important for inferring biological mechanisms from the patterns of distribution of sand flies in caves and their surroundings.

Therefore, cave environments represent an important setting for testing hypotheses about the patterns of distribution of sand fly faunas. This study aimed to test the hypothesis that there is high beta diversity between caves and their surroundings and, if indeed such high beta diversity exits, that it is driven more by species replacement than by abundance difference. This hypothesis is based on the fact that the peculiar characteristics of cave environments, such as little variation in temperature and humidity in its interior, lack light in areas distant from the entrance and overall oligotrophic conditions (Poulson and White 1969, Trajano and Gnaspini-Netto 1991) as well as, differences observed in activity rhythms among the cave interior, entrance and surrounding areas due to the presence of sunlight (Campos et al. 2016), presumably led to differences in the sand fly communities found within and surrounding caves.

Materials and Methods

Study area. The study included caves and their surroundings at three different locations in the state of Minas Gerais: Parque Estadual Serra do Rola Moça (PESRM), Moeda Sul and Parque Estadual Serra do Ouro Branco (PESOB) (Fig 1). These locations are within the region of the Quadrilátero Ferrífero of Minas Gerais, a region known to have a great number of recorded caves (Do Carmo et al. 2011, CECAV 2016). The region has abundant ferruginous fields called “canga”, a rare geological formation only found in the Quadrilátero Ferrífero of Minas Gerais and in Carajás, in the state of Pará (Da Silva et al. 1996, Jacobi and Do Carmo 2008).

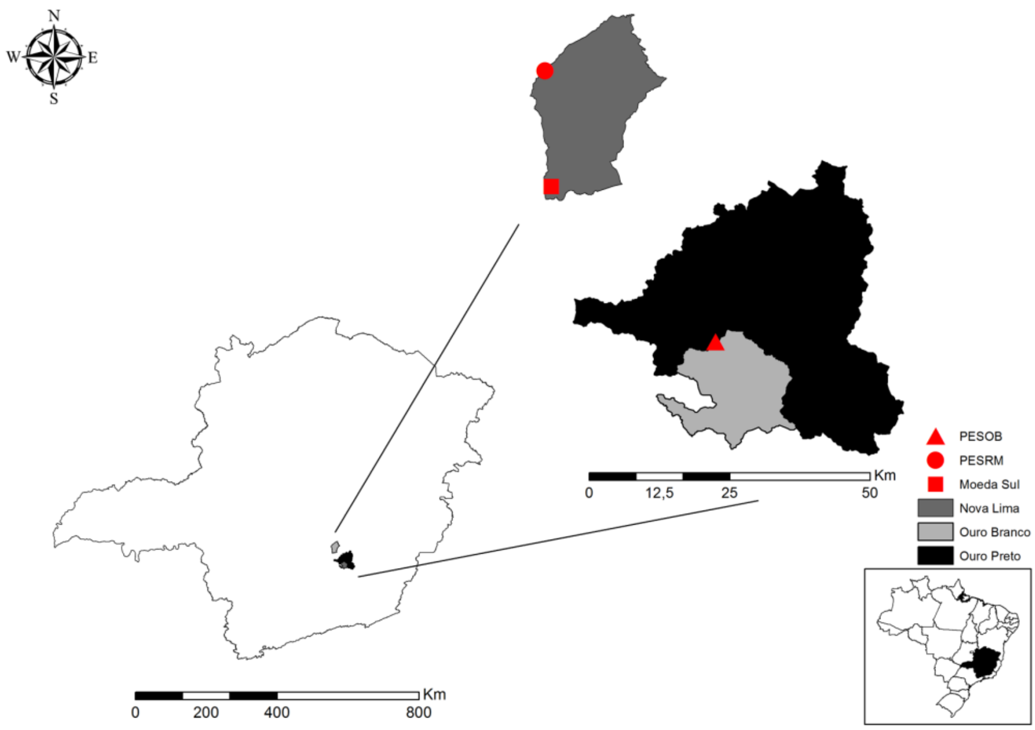

Fig 1. Location of the study area in the region of the Quadrilátero Ferrífero, Minas Gerais state, Brazil.

The study took place in five caves and their surroundings, three at PESRM (RM38, RM39 and RM40), one at Moeda Sul (MS) and one at PESOB (GI) (Table 1). All the caves were named by Centro Nacional de Pesquisa e Conservação de Cavernas (CECAV). The caves of PESRM sampled in this study are about 500 m from each other, and about 20 km from the caves of Moeda Sul (MS) and about 60 km from the cave at PESOB. The two caves RM39 and RM40 do not have an aphotic zone, whereas caves GI, MS and RM38 have aphotic zone about 5 m into the interior from the entrance.

Table 1. Description of caves sampled in Parque Estadual Serra do Ouro Branco, Moeda Sul and Parque Estadual Serra do Rola Moça, municipalities of Ouro Branco-MG, Ouro Preto-MG and Nova Lima-MG in the region of the Quadrilátero Ferrífero, from February 2014 to April 2015.

Description of caves				
Locations	Cave name	Coordinates	Elevation (m a.s.l)	Horizontal extension (m)
	RM38	20°00'48"S, 43°58'43"W	1,363	50
PESRM	RM39	20°00'56"S, 43°58'38"W	1,272	50
	RM40	20°00'59"S, 43°58'40"W	1,264	20
Moeda Sul	MS	20°12'7.19"S, 43°58'2.52"W	1,440	70
PESOB	GI	20°27'01"S, 43°42'16"W	1,219	1000

The caves of PESRM and Moeda Sul are all “canga” caves with no running water and are located in the municipality of Nova Lima in a transition zone between the Cerrado and Atlantic Forest biomes. The area is rich in ferruginous fields and high altitude fields beyond areas of “capoeira” (secondary formations) (Jacobi and Do Carmo 2008, IEF 2016). These caves are in a region of intense mining activity, and Moeda Sul continues to suffer from exploitation for real estate and urbanization (CECAV 2016). The cave sampled in PESOB consists of limestone and dolomitic marble with running water and is located in the municipality of Ouro Branco. The vegetation of this region is comprised of “campos rupestres” (rocky fields) and high altitude fields, gallery forests, patches of forest and remnants of Atlantic Forest (IEF 2016), and suffers impacts from mining.

Collection of sand flies. The collection of sand flies was performed under the license Nº 45636-1 of the Ministério do Meio Ambiente do Brasil and Nº 082/2014 of the Instituto Estadual de Florestas de Minas Gerais.

Systematic collections were performed using model HP automatic light traps, (Pugedo et al. 2005), throughout an entire year in PESRM (February 2014 to January 2015) and Moeda Sul (April 2014 to March 2015), and six months (September and October 2014 and from January to April 2015) in PESOB. Sampling took place monthly for three consecutive days for a total of 72 h of sampling effort per trap per month.

In PESRM, three traps were placed in RM38 cave, and six traps in its surroundings. For the caves RM39 and RM40 and their surroundings, two traps were placed in each cave and four in their surroundings, for a total of 21 traps (seven traps inside of caves and 14 in their surroundings). In Moeda Sul, eight traps were installed in one cave (MS) and eight in its surroundings for a total of 16 traps. In PESOB, six traps were installed in one cave (GI) and six in its surroundings for a total of 12 traps. For all caves and their surroundings sampled, the first trap inside a cave was at its entrance and the others were set every 10 m further into the cave. In the surroundings, the first trap was set in the forest 10 m from the cave entrance and the others were set every 10 m further away from the cave into the forest. The traps were installed approximately 1 m above the ground in all cave and surrounding environments.

In PESRM, we chose to install a different number of traps in the caves and in their surroundings due to small horizontal extension of the caves, making it impossible to use a greater number of traps. Thus, for a better sampling of sand fly fauna at this location we chose to double the number of traps in the surroundings of each cave.

After sampling, the traps were removed and the sand flies killed in glycerinated alcohol, sorted and sexed. Males were then placed in test tubes containing 70% alcohol and females were placed in test tubes containing 6% DMSO for future study of *Leishmania* DNA detection.

Sand fly identification. Male sand flies were prepared, mounted in Canada Balsam and identified following the classification of Galati (2003) and nomenclature of the species of the genus *Psychodopygus* follows the proposal of Carvalho et al. (2006). For female identification, part of the body (head and the last three segments of the abdomen) was separated and mounted in Berlese medium, with the head positioned ventrally. Specimens will be deposited in the Coleção de Flebotomíneos of the Centro de Pesquisas René Rachou/Fiocruz (FIOCRUZ/COLFLEB).

Statistical analysis. The partitioning of beta diversity between a cave and its surroundings of each site (GI, MS, RM39 and RM40) was calculated from the sum of species abundance data from all trapping points of each cave and its surroundings, divided by the number of exposed traps during every month of sampling using the total dissimilarity index of Sorensen (D_{totals}), species replacement index of Sorensen ($Repl_s$) and abundance difference (AbDiff) as proposed by Podani and Schmera (2011), Carvalho et al. (2012, 2013) and Podani et al. (2013), where $D_{totals} = Repl_s + AbDiff$. D_{totals} = total beta diversity, reflecting both species replacement and abundance difference; $Repl_s$ = beta diversity explained by species replacement; AbDiff = beta diversity explained by abundance difference. The partitioning of beta diversity was performed in the BAT package (Cardoso et al. 2015) in software R 3.2.4 (R Development Core Team 2016).

No phlebotomine sand fly was caught inside cave RM38, and so this cave and its surroundings were removed from the beta diversity partitioning analysis.

Results

A total of 722 phlebotomine sand flies were collected from 24 species and ten genera. The total of specimens by sex and the number of species in all environments (cave and surroundings) are shown in Table 2. The most abundant species was *Evandromyia tupynambai* (28%), followed by *Evandromyia edwardsi* (19%), *Br. troglodytes* (14%) and *Psychodopygus lloydi* (11%). Females of *Brumptomyia* represented 11% of the total, whereas all other species together amounted to 17% (Table 2).

Due to the morphological similarity of the genitalia of female sand flies of the genus *Brumptomyia*, and the occurrence of males of three species of this genus in the studied environments of the PESOB, species identification of females of this genus was difficult. Thus, males of *Brumptomyia* found in PESOB were identified to species but females were identified only to genus (i.e. *Brumptomyia* sp.).

Table 2. Phlebotomine sand flies collected in caves and their respective surroundings in Parque Estadual Serra do Ouro Branco, Moeda Sul and Parque Estadual Serra do Rola Moça, municipalities of Ouro Branco-MG, Ouro Preto-MG and Nova Lima-MG, respectively, from February 2014 to April 2015. "S" after the cave name indicates samples collected in the area surrounding the cave, and "CAV" indicates samples collected within the cave.

Species	PESOB							Moeda Sul							PESRM																			T
	GI_S			GI_CAV			T	MS_S			MS_CAV			T	RM38_S			RM38_CAV			RM39_S			RM39_CAV			RM40_S			RM40_CAV			T	
	♀	♂	T	♀	♂	T		♀	♂	T	♀	♂	T		♀	♂	T	♀	♂	T	♀	♂	T	♀	♂	T	♀	♂	T	♀	♂	T		
Brumptomyia carvalheiroi	-	11	11	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
Brumptomyia nitzulescui	-	23	23	-	3	3	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26
Brumptomyia sp.	69	-	69	14	-	14	83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	83
Brumptomyia troglodytes	-	2	2	-	-	-	2	-	-	-	-	-	-	-	4	-	4	-	-	-	23	16	39	6	3	9	13	23	36	5	3	8	96	98
Evandromyia edwardsi	11	3	14	64	33	97	111	1	-	1	-	-	-	1	-	-	-	-	-	-	1	-	1	4	17	21	-	-	-	-	-	-	22	134
Evandromyia evandroi	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Evandromyia termitophila	1	-	1	-	-	-	1	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Evandromyia tupynambai	-	-	-	1	-	1	1	-	1	1	-	-	-	1	-	-	-	-	-	-	2	1	3	66	42	108	-	-	-	42	51	93	204	206
Lutzomyia ischyracantha	-	-	-	-	-	-	-	-	1	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Lutzomyia longipalpis	1		1	2		2	3	1		1	4	1	5	6	-	-	-	-	-	-	-	-	-	1	1	2	-	1	1	3	4	7	10	19
Lutzomyia renei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Migonemyia migonei	-	1	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Micropygomyia quinquefer	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Nyssomyia whitmani	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1	1
Psathyromyia baratai	4	5	9	1	1	2	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
Psathyromyia brasiliensis	-	-	-	-	-	-	-	9	2	11	6	1	7	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18
Psathyromyia limai	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	1	1
Psathyromyia pascalei	2	4	6	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6

Continues

Table 1 – Continued

Species	PESOB							Moeda Sul							PESRM																			T
	GI_S			GI_CAV			T	MS_S			MS_CAV			T	RM38_S			RM38_CAV			RM39_S			RM39_CAV			RM40_S			RM40_CAV			T	
	♀	♂	T	♀	♂	T		♀	♂	T	♀	♂	T		♀	♂	T	♀	♂	T	♀	♂	T	♀	♂	T	♀	♂	T	♀	♂	T		
Pintomyia fischeri	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1	2
Pintomyia misionensis	2	-	2	-	-	-	2	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Pintomyia monticola	5	-	5	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	4	2	-	2	3	-	3	-	-	-	9	14
Psychodopygus carrerai	1	1	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Psychodopygus davisi	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Psychodopygus lloydi	60	17	77	1	-	1	78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	78
Sciopemyia sordellii	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total individuals per sex	158	67	225	83	39	122	347	12	4	16	11	3	14	30	5	-	5	-	-	-	30	18	48	79	63	142	18	24	42	50	58	108	345	722
Total species	12	9	16	6	5	9	19	4	3	6	3	3	4	8	2	-	2	-	-	-	3	3	5	5	4	5	4	2	5	3	3	3	9	24

Localities: PESOB=Parque Estadual Serra do Ouro Branco, Moeda Sul, PESRM=Parque Estadual Serra do Rola Moça; Environments: GI_S and GI_CAV=Gruta da Igrejinha surroundings and cave, MS_S and MS_CAV=Moeda Sul surroundings and cave, RM38_S and RM38_CAV= Rola Moça 38 surroundings and cave, RM39_S and RM39_CAV=Rola Moça 39 surroundings and cave, RM40_S and RM40_CAV=Rola Moça 40 surroundings and cave. T=total.

In PESOB, 347 individuals were collected with 122 from inside cave Gruta da Igrejinha (GI) and 225 from its surroundings. The most abundant species in the cave was *Ev*. *edwardsi* (79%), and the most abundant in its surroundings was *Ps*. *lloydi* (34%). In Moeda Sul, 30 individuals were collected, with 16 from inside the cave MS and 14 from its surroundings, with the most abundant species in the cave being *Psathyromyia brasiliensis* (50%) and *Lutzomyia longipalpis* (36%), whereas in the surroundings the most abundant was *Pa*. *brasiliensis* (69%). In PESRM, only five individuals were collected from the surroundings of cave RM38 (none from inside the cave); these were *Br*. *troglodytes* (80%) and *Lutzomyia renei*. Inside cave RM39 and from its surroundings a total of 190 individuals were collected (48 in the cave and 142 from its surroundings), with *Ev*. *tupynambai* (76%) being the most abundant species in the cave, and *Br*. *troglodytes* (81%) the most abundant in its surroundings. Inside cave RM40 and from its surroundings a total of 150 individuals were collected (42 in the cave and 108 in its surroundings) with *Ev*. *tupynambai* (86%) being the most abundant in the cave and *Br*. *troglodytes* (86%) the most abundant in its surroundings (Table 2).

The beta diversity between each cave and its surroundings was high and determined both by species replacement and abundance difference. At Gruta da Igrejinha (GI), beta diversity was determined mostly by species replacement and less by abundance difference. At Moeda Sul (MS), beta diversity was determined only by species replacement, whereas at Rola Moça 39 (RM39) and Rola Moça 40 (RM40) it was mostly determined by abundance difference (Fig 2). The contributions of species replacement and abundance difference between caves and their surroundings at Moeda Sul, Rola Moça 39 and Rola Moça 40 were quite different, whereas at Gruta da Igrejinha the difference in their contributions was smaller (Fig. 2).

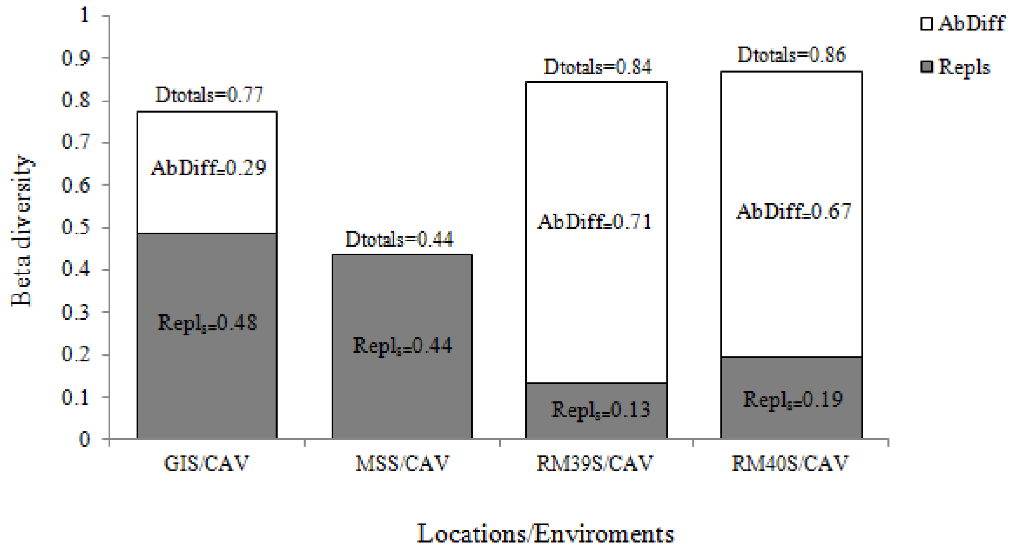

Fig 2. Partitioning of total beta diversity (D_{totals}) into species replacement ($Repl_{ls}$) and abundance difference (AbDiff) between the cave and its surroundings at each sampling locality. Location/Environment: GIS and GICAV= Gruta da Igrejinha surroundings and cave, MSS and MSCAV=Moeda Sul surroundings and cave, RM39S and RM39CAV= Rola Moça 39 surroundings and cave, RM40S and RM40CAV= Rola Moça 40 surroundings and cave.

The beta diversity determined by species replacement and abundance difference between each cave and its surrounding was related to the number of exclusive species plus the abundance difference between species and with the difference in the total abundance of the species between these two environments, respectively (Table 3).

Table 3. Individuals number and of exclusive and common sandfly species collected in collected cave environments and their respective surroundings and both environments in Parque Estadual Serra do Ouro Branco, Moeda Sul and Parque Estadual Serra do Rola Moça, municipalities of Ouro Branco-MG, Ouro Preto-MG and Nova Lima-MG, respectively, from February 2014 to April 2015. "S" after the cave name indicates samples collected in the area surrounding the cave, and "C" indicates samples collected within the cave.

	PESOB			Moeda Sul			PESRM					
	GI			MS			RM39			RM40		
	GI_S	GI_CAV	GI_S_CAV	MS_S	MS_CAV	MS_S_CAV	RM39_S	RM39_CAV	RM39_S_CAV	RM40_S	RM40_CAV	RM40_S_CAV
Brumptomyia carvalheiroi	X	-		-	-	-	-	-	-	-	-	-
Brumptomyia nitzulescui	-	-	X	-	-	-	-	-	-	-	-	-
Brumptomyia sp.	-	-	X	-	-	-	-	-	-	-	-	-
Brumptomyia troglodytes	X	-	-	-	-	-	-	-	X	-	-	X
Evandromyia edwardsi	-	-	X	X	-	-	-	-	X	-	-	-
Evandromyia evandroi	-	-	-	-	X	-	-	-	-	-	-	-
Evandromyia termitophila	X	-	-	-	X	-	-	-	-	-	-	-
Evandromyia tupynambai	-	X	-	X	-	-	-	-	X	-	X	-
Lutzomyia ischyracantha	-	-	-	X	-	-	-	-	-	-	-	-
Lutzomyia longipalpis	-	-	X	-	-	X	-	X	-	-	-	X
Lutzomyia renei	-	-	-	-	-	-	-	-	-	-	-	-
Migonemyia migonei	X	-	-	-	-	-	-	-	-	-	-	-
Micropygomyia quinquefer	-	X	-	-	-	-	-	-	-	-	-	-
Nyssomyia whitmani	-	-	-	-	-	-	-	-	-	X	-	-
Psathyromyia baratai	-	-	X	-	-	-	-	-	-	-	-	-

Continues

Table 3 – Continued

Espécies	PESOB			Moeda Sul			PESRM					
	GI			MS			RM39			RM40		
	GI_S	GI_CAV	GI_S_CAV	MS_S	MS_CAV	MS_S_CAV	RM39_S	RM39_CAV	RM39_S_CAV	RM40_S	RM40_CAV	RM40_S_CAV
Psathyromyia brasiliensis	-	-	-	-	-	X	-	-	-	-	-	-
Psathyromyia limai	-	-	-	-	-	-	X	-	-	-	-	-
Psathyromyia pascalei	X	-	-	-	-	-	-	-	-	-	-	-
Pintomyia fischeri	X	-	-	-	-	-	-	-	-	X	-	-
Pintomyia misionensis	X	-	-	X	-	-	-	-	-	-	-	-
Pintomyia monticola	X	-	-	-	-	-	-	-	X	X	-	-
Psychodopygus carrerai	X	-	-	-	-	-	-	-	-	-	-	-
Psychodopygus davisi	X	-	-	-	-	-	-	-	-	-	-	-
Psychodopygus lloydi	-	-	X	-	-	-	-	-	-	-	-	-
Sciopemyia sordellii	-	X	-	-	-	-	-	-	-	-	-	-
Total individuals	32	3	312	4	2	24	1	2	187	5	93	52
Total species	10	3	6	4	2	2	1	1	4	3	1	2
Percentage individuals	9%	1%	90%	13%	7%	80%	0.5%	1%	98.5%	3%	62%	35%
Percentage species	52.6%	15.8%	31.6%	50%	25%	25%	17%	17%	66%	50%	17%	33%

PESOB, Moeda Sul, PESRM, GI_S and GI_CAV, MS_S and MS_CAV, RM39_S and RM39_CAV and RM40_S and RM40_CAV have the same legend of table 2.

GI_S_CAV, MS_S_CAV, RM39_S_CAV, RM40_S_CAV= surroundings plus cave of the Gruta da Igrejinha, Moeda Sul, Rola Moça 39 and Rola Moça 40, respectively.

Discussion

The original hypothesis of this study that there is high beta diversity between caves and their surroundings being driven more by species replacement than by abundance difference was partially corroborated by the results. We did find high beta diversity between caves and their surroundings, but we did not observe more species replacement than abundance difference for all locations studied (GI, MS, RM39 and RM40).

The greatest influence of species replacement over that of abundance difference between a cave and its surroundings was for cave GI, and there was only species replacement (i.e., no abundance difference) between the two environments for MS, illustrating the predominance of exclusive species plus the abundance difference between species to one or the other of these environments, as has been reported previously Legendre (2014), and the small (GI) or lack of (MS) differences in the total abundance of species between these two environments. However, at caves RM39 and RM40, there was the greatest influence of abundance difference over that of species replacement, illustrating that the cave and surrounding communities differ mostly in the total abundance of the species, as was previous reported by Legendre (2014).

The caves of GI and MS have an aphotic zone that extends for almost their entire length, and so this condition in these caves could have limited to distribution and colonization of sand flies between these caves and their surroundings, preventing some species from inhabiting both of these environments or a larger survival of individuals in an environment other than and resulting in greater species replacement. According to Culver (1982), the peculiar characteristics of the cave environment, including oligotrophic conditions, stability of climate, and the absence of light, act as limiting factors for the arrival and establishment of new species. Thus, theoretically, these peculiar conditions of the caves can favor the predominance of phlebotomine sand fly species tolerant to these conditions, while serve as limiting factors to other species. Working with other invertebrates in caves with a lack of interior light, Prous et al. (2015) found that the similarity among cave faunas decreased gradually from the exterior to the entrance, reaching values of zero in the cave interior, suggesting that the interior of the caves were inhabited by an entirely unique invertebrate community.

Caves RM39 and RM40 did not have an aphotic zone, which therefore may have favored the occurrence of sand flies moving between these caves and their surroundings resulting in just a few individuals being replaced between different species, and most differences being in the total abundance of species between these two environments (almost all species were most abundant in an environment than another) resulting in a higher proportion of beta diversity by abundance difference. These results suggest that sand flies may search caves for protection, shelter, reproduction and availability of resources, since the cave had greater total abundance of species than its surroundings. According to Arias et al. (1985) and Galati et al. (2003c), temperature, humidity, altitude, rainfall, luminosity, vegetation and availability of resources influence the establishment of phlebotomine sand fly colonies or make environments favorable for the resting and breeding of these insects, since the immature forms develop in moist soil that is protected from direct incidence of light and wind, and rich in organic matter (Forattini 1973, Galati et al. 2003c).

Some studies have reported that beta diversity caused by species replacement may be due to differences in environmental conditions, biotic interactions, area, habitat richness, proportion of endemism, elevation, distances between areas (Baselga 2008, Hirao et al. 2015, Si et al. 2015, Boschilia et al. 2016), while beta diversity by abundance difference may be due to diversity of niches available and other ecological processes (Legendre 2014).

The phlebotomine sand fly fauna collected inside and outside of the studied caves was very diverse, with species restricted to cave environments, other species restricted to cave surroundings and others common to both environments. The finding of a rich sand fly fauna in cave environments has been reported previously by Carvalho et al. (2013) who found 29 species of sand flies in caves in the municipality of Lassance, state of Minas Gerais. Galati et al. (2010) collected 11 species in caves in the state of São Paulo and Barata et al. (2008) collected 14 species in caves located in Parque Nacional do Peruaçu, state of Minas Gerais.

In conclusion, beta diversity between each cave and its surroundings was high. In caves that had an aphotic zone beta diversity was determined mostly by species replacement and in cave that did not have an aphotic zone beta diversity was mostly determined by abundance difference. Therefore, with these results we suggest that both caves and their surroundings are important for the maintenance of their sand fly communities and that the peculiar characteristics of cave environments has presumably led to differences in the sand fly communities within caves and in their surroundings.

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References Cited

Alves, V. R., R. A. Freitas, and T. V. Barrett. 2008. *Lutzomyia maruaga* (Diptera: Psychodidae), a new bat-cave sand fly from Amazonas, Brazil. Mem. Inst. Oswaldo Cruz. 103: 251-253.

Alves, V. R., R. A. De Freitas, F. L. Santos, and T. V. Barrett. 2011. Diversity of sandflies (Psychodidae: Phlebotominae) captured in sandstone caves from Central Amazonia, Brazil. Mem. Inst. Oswaldo Cruz. 106: 353–359.

Barata, R. A., Y. Antonini, C. M. Gonçalves, D. C. Costa, and E. S. 2008. Dias. Flebotomíneos do Parque Nacional Cavernas do Peruaçu, MG. Neotrop Entomol. 37: 226–228.

Baselga, A. 2008. Determinants of species richness, endemism and turnover in European longhorn beetles. Ecography. 31: 263–271.

Boschilia, S. M., De Oliveira E. F., and A. Schwarzbold. 2016. Partitioning beta diversity of aquatic macrophyte assemblages in a large subtropical reservoir: prevalence of turnover or nestedness?. Aquat Sci. 78: 615–625.

Cardoso, P., Rigal F., and J. C. Carvalho. 2015. BAT – Biodiversity Assessment Tools, an R package for themeasurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. Met. Ecol. Evol. 6: 232–236.

Carvalho, G. M. L., A. L. Falcão, and J. D. Andrade Filho. 2006. Taxonomic revision of phlebotomine sand fly species in the series *davisi* and *panamensis* of the subgenus *Psychodopygus* Mangabeira, 1941 (Diptera: Psychodidae: Phlebotominae). Mem Inst Oswaldo Cruz. 101: 129–136.

Carvalho, G. M. D. L., R. P. Brazil, C. C. Sanguinette, and J.D. Andrade Filho. 2011. Description of *evandromyia spelunca*, a new phlebotomine species of the cortelezzii complex, from a cave in Minas Gerais State, Brazil (Diptera: Psychodidae: Phlebotominae). Parasit Vectors 4 (158): 1-8.

Carvalho, G. M. L., R. P. Brazil, L. Saraiva, P. F. Quaresma, H. A. Botelho, M. C. N. F. Da Ramos, A. P. L. A. Zenóbio, P. C. L. S. Meira, C. C. Sanguinette, and J. D. Andrade Filho. 2012. Hourly Activity and Natural Infection of Sandflies

(Diptera: Psychodidae) Captured from the Aphotic Zone of a Cave, Minas Gerais State, Brazil. Plos One 7 (12): 1-6.

Carvalho, J.C., P Cardoso, and P. Gomes, P. 2012. Determining the relative roles of species replacement and species richness differences in generating beta-diversity patterns. Global Ecol. Biogeogr. 21: 760–771.

Carvalho, G. M. D. L., R. P. Brazil, M. C. D. N. F. Ramos, P. C. L. Serra and Meira, A. P. L. D. A. Zenóbio, H. A. Botelho, C. C. Sanguinette, L. Saraiva, and J. D. Andrade Filho. 2013. Ecological Aspects of Phlebotomine Sandflies (Diptera: Psychodidae) from a Cave of the Speleological Province of Bambuí, Brazil. Plos One 8 (10): 1–10.

Campos, A. M. , R. Matavelli, C. L.C. D. Santos, L. S. Moraes, and J. M. M. Rebêlo. 2013. Ecology of phlebotomines (Diptera: Psychodidae) in a transitional area between the amazon and the cerrado in the state of maranhão, Brazil. J Med Entomol. 50: 52–58.

Campos, A. M., C. L. C. Dos Santos, R. Stumpp, L. H. D. Da Silva, R. A Maia, A. P. Paglia, and J. D. Andrade Filho. 2016. Photoperiod differences in sand fly (Diptera: Psychodidae) species richness and abundance in caves in Minas Gerais State, Brazil. J Med Entomol. 135: 1-6.

CECAV. 2016. Centro Nacional de Pesquisa e Conservação de Cavernas. 2016. Base de Dados de Cavidades Naturais. (http://www.icmbio.gov.br/cecav/downloads/mapas.html) (accessed 10 April 2016).

Culver, D. C. 1982. Cave Life. Evolution and Ecology. Press HU, editor. Cambridge, Massachussets and London, England.

Da Silva, M. F. F., R. S. Secco, and M. G. A. Lobo. 1996. Aspectos ecologicos da vegetação rupestre da Serra dos Carajás, Estado do Pará, Brasil. Acta Amaz. 26: 16–44.

Do Carmo, F. F., F. F. Do Carmo, A. A. R. Salgado, and C. M. Jacobi. 2011. Novo sítio espeleológico em sistemas ferruginosos, no Vale do Rio Peixe Bravo, norte de Minas Gerais , Brasil. Espeleo-tema. 22.

Ferreira, R. L., and L. C. Horta. 2001. Natural and human impacts on invertebrate communities in Brazilian caves. Braz J Biol. 61: 7–17.

Ferreira, R.L., L. Ferreira, D. O. Bernardi, and M. S. Silva. 2009. Caracterização dos ecossistemas das Grutas Aroê Jari , Kiogo Brado e Lago Azul (Chapada dos Guimarães, MT): Subsídios para o turismo nestas cavidades. Rev Biol e Ciências da Terra. 9: 41–58.

Forattini, O. P. 1973. Entomologia Médica. Phlebotominae. Leishmanioses e Bartoneloses. Ltda EB, editor, São Paulo, SP.

Galati, E. A. B. 2003. Morfologia e taxonomia. Classificacão de Phlebotominae. *In* EF. Rangel, R. Lainson (org.), 1st ed., Flebotomíneos do Brasil, Fiocruz, Rio de Janeiro, p. 23–51.

Galati, E. A. B., J. D. Andrade Filho, A. C. L. Silva, and A. L. Falcão. 2003a. Description of a new genus and new species of New World Phlebotominae (Diptera, Psychodidae). Rev. Bras. Entomol. 47: 63-70.

Galati, E. A. B., A. M. Marassá, and R. M. G. Andrade. 2003b. *Micropygomyia* (*Sauromyia*) *petari*, a new species of Phlebotominae (Diptera: Psychodidae) from Vale do Ribeira, São Paulo state, Brazil. Rev. Bras. Entomol. 47: 455-459.

Galati, E. A. B., V. L. B. Nunes, P. C. Boggiani, M. E. C. Dorval, G. Cristaldo, H. C. da Rocha, E. T. Oshiro, R. M. Gonçalves-de-Andrade, and G. Naufel. 2003c. Phlebotomines (Diptera: Psychodidae) in caves of the Serra da Bodoquena, Mato Grosso do Sul State, Brazil. Rev. Bras. Entomol. 47: 283–296.

Galati, E. A. B., V. L. B. Nunes, P. C. Boggiani, M. E. C. Dorval, G. Cristaldo, H. C. da Rocha, E. T. Oshiro, R. M. Gonçalves-de-Andrade, and G. Naufel. 2006. Phlebotomines (Diptera: Psychodidae) in forested areas of the Serra da Bodoquena, state of Mato Grosso do Sul, Brazil. Mem Inst Oswaldo Cruz. 101: 175–193.

Galati, E. A. B., A. M. Marassá, R. M. G Andrade, E. F. M. Bueno, B. R. Paiva, and R. S. Malafronte. 2010. *Nyssomyia intermedia* (Lutz & Neiva) and *Nyssomyia neivai* (Pinto) (Diptera, Psychodidae, Phlebotominae) in a sympatric area: seasonal and nocturnal hourly rhythm in black and white modified Shannon traps. Rev. Bras. Entomol. 54: 677–686.

Hirao, T., Kubota, Y. and Murakami M. 2015. Geographical patterns of butterfly species diversity in the subtropical Ryukyu Islands: the importance of a unidirectional filter between two source islands. J. Biogeogr. 42, 1418–1430.

IEF. 2016. Instituto Estadual de Florestas. (http://www.ief.mg.gov.br/). (accessed 10 July 2016).

Jacard, P. 1912. The distribution of the flora in the alpine zone. New Phytol. 11: 37–50.

Jacobi, C. M., and F. F. Do Carmo. 2008. Diversidade dos campos rupestres ferruginosos no Quadrilátero Ferrífero, MG. Megadiversidade. 4: 25–33.

Legendre, P. 2014. Interpreting the replacement and richness difference components of beta diversity. Global Ecol. Biogeogr. 23: 1324–1334.

Leprieur, F., , P.A. Tedesco, B. Hugueny, O. Beauchard, H.H. Dürr, S. Brosse, and T. Oberdorff. 2011. Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. Ecol. Letters. 14. 325–334.

Matavelli, R., A. M. Campos, R. N. Feio, and R. L. Ferreira. 2015. Occurrence of anurans in brazilian caves. Acta Carsologica. 44: 107–120.

Podani, J., and D. Schmera. 2011. A new conceptual and methodological framework for exploring and explaining pattern in presence–absence data. Oikos. 120: 1625–1638.

Podani, J., C. Ricotta, and D. Schmera. 2013. A general framework for analyzing beta diversity, nestedness and related community-level phenomena based on abundance data. Ecol. Complex. **15**: 52–61.

Poulson, T. L., and W. B. White. 1969. The Cave Environment. Science 165: 971–981.

Prous, X., R.L. Ferreira, and C. M. Jacobi. 2015. The entrance as a complex ecotone in a Neotropical cave. Int J Speleol. 44: 177–189.

Pugedo, H., R. A. Barata, J. C. França-Silva, J. C. Silva, and E. S. Dias. 2005. HP: um modelo aprimorado de armadilha luminosa de sucção para a captura de pequenos insetos. Rev. Soc. Bras. Med. Trop. 38: 70–72.

Qian, H., R. E. Ricklefs, and P. S. White. 2005. Beta diversity of angiosperms in temperate floras of eastern Asia and eastern North America. Ecol Lett. 8: 15–22.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. Available at http://www.R-project.org.

Saraiva, L., R. A Silva, N. R. J. Marteleto, P. A. A Sampaio, F. D. Rêgo, M. R. A. C. Vianna, C. M. F. Gontijo, and J. D. Andrade Filho. 2015. Survey of Sand Flies (Diptera: Psychodidae) in an Environmentally Protected Area in Brazil. Plos One 10: 1–5.

Si, X., A. Baselga, and P. Ding. 2015. Revealing Beta-Diversity Patterns of Breeding Bird and Lizard Communities on Inundated Land-Bridge Islands by Separating the Turnover and Nestedness Components. Plos One. 1-19.

Simpson, G. G. 1943. Mammals and the nature of continents. Am J Sci. 241: 1–31.

Sørensen, T.A. 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content, and its application to analyses of the vegetation on Danish commons. K Danske Vidensk Selsk Biol Skr. 5: 1–34.

Trajano E, and Gnaspini-Netto P. 1991. Composição da fauna cavernícola brasileira, com uma análise preliminar da distribuição dos taxons. Rev Bras Zool. 7: 383–407.

Considerações finais

Lutzomyia renei foi à espécie mais abundante dentro das cavernas durante os períodos diurno e noturno, mostrando o aspecto troglófilo dessa espécie nesses ambientes. *Lutzomyia longipalpis*, espécie com grande importância vetorial no Brasil, foi à segunda espécie mais abundante na caverna durante os períodos diurno e notuno representando um possível risco de transmissão de *Leishmania* entre os reservatórios e humanos que frequentam esses ambientes.

Verificamos que o período de atividade diária dos flebotomíneos nos ambientes de caverna é afetado diretamente pela presença da luz solar, visto que nossos resultados mostraram que os flebotomíneos exibem diferença nos seus ritmos circadianos entre os ambientes do entorno, entrada (zona fótica) e interior (zona afótica) das cavernas. No interior das cavernas, observamos que a riqueza e abundância do total de flebotomíneos e a abundância de machos e fêmeas de flebotomíneos não diferiu entre os períodos diurno e noturno, mas diferiu entre os períodos na entrada e entorno das cavernas, com maior riqueza e abundância do total de flebotomíneos e a abundância de machos e fêmeas durante o período noturno. Esses resultados mostram que os flebotomíneos coletados na entrada e entorno das cavernas respondam a um ciclo circadiano noturno, enquanto que no interior das cavernas esses insetos estão ativos em ambos os períodos.

Esses resultados sobre o período de atividade dos flebotomíneos em cavernas (entrada e interior) e seus entornos mostram a importância em se investigar o papel de vertebrados nesses ambientes como possíveis fontes alimentares sanguínea desses insetos e/ou reservatório de *Leishmania* e na manutenção da fauna flebotomínica ativa em ambos os períodos dentro de cavernas. O número elevado de fêmeas capturadas na entrada e interior das cavernas e ativas em ambos os períodos no seu interior, suportam a hipótese de que vertebrados possam estar funcionando como fonte alimentar sanguínea para esses insetos. Esse achado chama atenção, visto que esses insetos se alimentam em vertebrados e, portanto, podem transmitir *Leishmania*, o agente causador das leishmanioses e assim estabelecer um ciclo de transmissão de *Leishmania* entre os reservatórios e humanos que visitam esses ambientes.

Portanto, é importante o estudo de fonte alimentar sanguínea das fêmeas de flebotomíneos e de detecção de DNA de *Leishmania* nos vertebrados encontrados dentro e fora das cavernas e nas fêmeas de flebotomíneos coletadas nesses mesmos ambientes durante os mesmos períodos de coletas, visto que coletamos dentro e fora dessas cavernas espécies de flebotomíneos envolvidas na transmissão de *Leishmania*.

Encontramos alta diversidade beta entre caverna e seu entorno resultante de substituição de espécies e diferença de abundância, o que nos levou a pensar que as condições peculiares dos ambientes de cavernas possam ter influenciado a distribuição dos flebotomíneos encontrada dentro e fora das cavernas. Diante desse resultado, sugerimos que em estudos futuros se investigue o efeito de variáveis ambientais, tais como gradiente de temperatura, umidade e luminosidade, extensão horizontal e presença de vertebrados dentro das cavernas, assim como o efeito da paisagem dos seus entornos (cobertura e perda de vegetação) sobre a distribuição dos flebotomíneos presentes nesses ambientes.

Estudos em cavernas e seus entornos com essa abordagem de partição da diversidade beta em substituição de espécies e diferença de riqueza ou abundância são importantes para que se possa verificar a presença de algum padrão de distribuição dos flebotomíneos entre caverna e seu entorno como foi observado em nossos resultados.

Contudo, acreditamos que nossos resultados possam se repetir em outras cavernas e seus respectivos entornos, visto que são as condições peculiares dos ambientes cavernícolas que podem está modulando a distribuição e atividade diária dos flebotomíneos nesses ambientes.