ANNOTATED LIST OF SPECIES

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An updated list of sand flies (Diptera, Psychodidae, Phlebotominae) in the Federal District of Brazil

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Abstract

The Federal District of Brazil (FD) has the lowest Phlebotominae species richness in the country's Midwestern Region. Some of these species have been reported as *Leishmania* vectors. This study updates the list of sand flies in the FD by sampling 4 gallery forests. Using HP light traps and Shannon traps, 1,209 sand flies were captured and 16 species were identified. The most frequent species was *Bichromomyia flaviscutellata* (Mangabeira, 1942) (*N* = 668), followed by *Psathyromyia pradobarrientosi* (Le Pont, Matias, Martinez & Dujardin, 2004) (*N* = 285). *Brumptomyia guimaraesi* (Coutinho & Barretto, 1941), *Micropygomyia ferreirana* (Martins, Barretto & Pellegrino, 1956), *Pa. pradobarrientosi*, *Br. brumpti* (Larrousse, 1920), and *Evandromyia bourrouli* (Barretto & Coutinho, 1941) are reported for the first time in the FD. Sand flies richness in the FD (35 spp.) is relevant when compared to that of the state of Goiás (47 spp.), a state 59 times larger than the FD.

Key words

Psathyromyia pradobarrientosi; Brazilian savannah; species richness; list of species.

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Introduction

Phlebotomine sand flies (Diptera, Psychodidae, Phlebotominae) are insects with a wide geographic distribution where they are found in diverse climatic conditions and at a range of altitudes, as well as in rural, urban, and sylvatic environments. There are currently 1003 species, 537 of which are found in the Neotropical and Nearctic regions, and 277 in Brazil (Shimabukuro et al. 2017). Only 19 sand fly species have been incriminated as vectors of *Leishmania* species (Kinetoplastida, Trypanosomatidae), which may be etiological agents of cutaneous and visceral leishmaniasis (Shimabukuro and Galati 2011).

Most cases of leishmaniasis in the Americas originated in Brazil (Alvar et al. 2012). Between 2001 and 2012, approximately 3000 confirmed cases of visceral leishmaniasis (VL) and 50,000 of cutaneous leishmaniasis (CL) were reported in the Center-West Region of Brazil (http://www.saude.gov.br), where at least 127 phlebotomine species have been recorded (Almeida et al. 2015). This region is largely within the Brazilian Cerrado biome. In this savannah region, vast agricultural expansion, as well as occupation of the land due to unplanned urban growth may favor the occurrence of leishmaniasis (Galati et al. 1996, Carranza-Tamayo et al. 2010).

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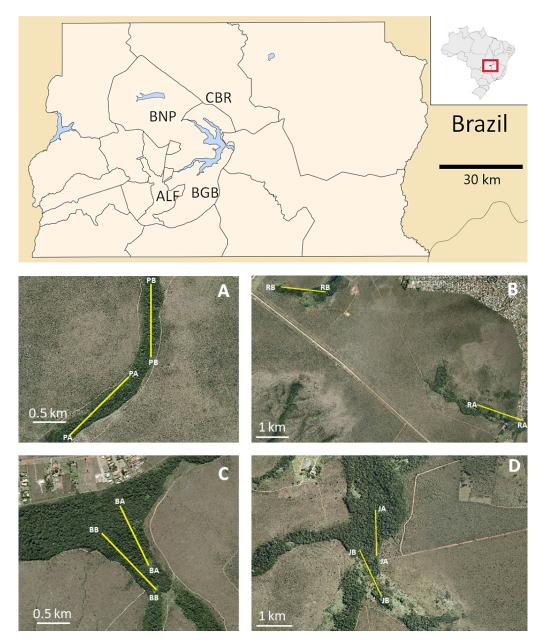


Figure 1. Geographic location of the four areas of the study performed in the Federal District of Brazil. **A.** BNP = Brasília National Park (trapping lines PA, PB). **B.** CBR = Contagem Biological Reserve (RA, RB). **C.** ALF = Água Limpa Farm within the University of Brasília (BA, BB). **D.** BGB = Brasília Botanical Garden (JA, JB). Source: CODEPLAN (Companhia de Planejamento do Distrito Federal2017 (http://ortofoto.mapa. codeplan.df.gov.br/demo/tms).

In the Distrito Federal do Brasil (Brazilian Federal District, hereinafter FD), there is evidence of autochthonous transmission of CL (Sampaio et al. 1980). The number of cases reported as originating from the FD in 2003, 2004, and 2005 were 31, 57, and 26, respectively (Sampaio et al. 2009). The first autochthonous case of VL in the FD occurred in 2005, and 3 deaths were confirmed by 2009 (Carranza-Tamayo et al. 2010). Between 2007 and 2012, 24 human cases of autochthonous VL were reported (data from Health Secretariat of the Federal District). In 2013, 98 suspect cases of VL were reported in the Brazilian National Notifiable Diseases Surveillance System (SINAN/NET), from which 47 were confirmed. Two of these cases-from the FD administrative regions of Sobradinho and Lago Norte-were autochthonous, and 1 case resulted in death. VL cases have also been documented in domestic dogs in northern and southern areas of the FD, which has experienced unplanned urbanization of rural areas (Carranza-Tamayo et al. 2010).

An understanding of the distribution of phlebotomine species is essential for the prevention of leishmaniasis (Cutolo et al. 2013); however, knowledge on their geographic distribution in the FD is still poorly understood, and the phlebotomine fauna has rarely been sampled or compared to that of other regions. Studies analyzing the occurrence of sand flies in FD are scarce (Carvalho et al. 2010, Ferreira et al. 2015), which hinders the identification of areas at risk for *Leishmania* spp. transmission and epidemiological policies to prevent leishmaniasis. In order to update the list of phlebotomine species occurring in the FD, we studied the sand fly fauna from gallery forests.
 Table 1. Institutional catalog number of the vouchers of phlebotomine species captured in this study.

Species (species code)	Catalog no. of the vouchers				
Bichromomyia flaviscutellata (Bif)	CBRBif 1 – 632				
	BNPBif 1 – 36				
Brumptomyia brumpti (Brb)	ALFBrb 1 – 2				
Brumptomyia guimaraesi (Brg)	ALFBrg 1 – 29				
	BGBBrg 1 – 2				
<i>Brumptomyia</i> sp. (Brsp)	ALFBrsp 1 – 41				
Evandromyia bourrouli (Evb)	ALFEvb 1 – 7				
	CBREvb 1 – 10				
	BGBEvb 1				
Micropygomyia longipennis (Mil)	ALFMil 1 – 3				
	CBRMil 1 – 7				
Micropygomyia ferreirana (Mif)	CBRMif 1 – 4				
Micropygomyia sp. (Misp)	ALFMisp 1				
Nyssomyia whitmani (Nyw)	CBRNyw 1 – 10				
	BNPNyw 1 – 16				
	BGBNyw 1				
Psathytromyia abonnenci (Paab)	ALFPaab 1				
	BNPBi 1 – 5				
Psathyromyia aragaoi (Paar)	ALFPaar 1				
Psathyromyia bigeniculata (Pab)	ALFPab 1 – 41				
	BNPPab 1				
	BGBPab 1				
Psathyromyia lutziana (Pal)	ALFPal 1 – 17				
	BNPPal 1				
	BGBPal 1				
Psathyromyia pradobarrientosi (Pap)	ALFPap 1 – 276				
	CBRPap 1				
	BNPPap 1 – 5				
	BGBPap 1 – 3				
Pintomyia christenseni (Pic)	ALFPic 1 – 26				
Pintomyia kuscheli (Pik)	ALFPik 1				
<i>Pintomyia monticola</i> (Pim)	ALFPim 1 – 24				
Sciopemyia sordellii (Scs)	ALFPic 1 – 3				

Methods

Study site. The Brazilian Federal District is located in the Center-West Region of Brazil. Its total area is 5,801,937 km², and it currently has a population of nearly 3 million inhabitants (IBGE 2016). The climate is classified as tropical according to the Köppen system, and it has 2 defined seasons: the rainy season (from October to April) and the dry season, which corresponds to the months of May to September (Klink and Machado 2005, Carvalho et al. 2010). The study was performed in four areas: the Brasília National Park (BNP), the Água Limpa Farm (ALF), the Contagem Biological Reserve (CBR), and the Botanical Garden of Brasília (BGB), which are shown in Figure 1.

BNP ($15^{\circ}41'51''S$, $047^{\circ}57'32W$) is the most important protected area in the FD, occupying an area of 30,566 ha (Parna 1997). ALF ($15^{\circ}57'17''S$, $047^{\circ}58'30''$ W) is the University of Brasília farm where agricultural and ecological research projects are conducted. Its area is approximately 4340 ha, 3140 ha of which are protected areas and 1200 ha are used for production. The BGB ($15^{\circ}53'17''S$, $047^{\circ}50'33''W$) occupies an area of 4518 ha, 526 ha of which are open to public visitation (Fon**Data collection.** In each gallery forest, 2 trap lines of 1 km each were established (Fig. 1), with 20 HP light traps (Pugedo et al. 2005) set up. They were spaced 50 m apart and left for 4 consecutive days in May and September 2014. One Shannon trap was set 50 m away from each trapping line. The total capture effort included 1280 HP traps (160 per area in each month) and 16 Shannon traps (4h). The selection of these periods was based on previous studies, which reported a more frequent occurrence of Phlebotominae in the dry months in the FD (Ferreira et al. 2015).

All specimens captured were conditioned in a 70% ethanol solution. The specimens were dissected, and the head and genitalia were clarified and mounted in Canada balsam between the slide and the cover slip for species identification (Galati 2015). The remaining body parts (the thorax, part of the abdomen, wings, and legs) were placed in Eppendorf tubes containing phosphate buffered saline (PBS) (1 body part per tube). The tubes were labeled and placed in the freezer. Some of the specimens were identified only to the genus level due to the integrity of the material. Codes of each specimen collected were based on the names of the studies areas (ALF: Água Limpa Farm, CRB: Contagem Biological Reserve, BNP: Brasília National Park, BGB: Botanical Garden of Brasília) followed by abbreviations of the genera and of the species name.

Data analyses. The frequencies of traps that were positive and negative for sand flies in each gallery forest and month were compared using Fisher's exact test and a significance level of 5%. Relative species abundance was also calculated for the sand flies identified.

Results

The total phlebotomine sample from the 4 gallery forests in May and September 2014 consisted of 1209 specimens (615 males, 594 females) and 16 species. All vouchers were deposited in the Faculty of Medicine of the University of Brasilia (Table 1).

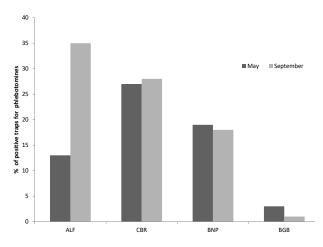
The greatest number of species was recorded in ALF and the greatest number of specimens was captured in CBR. Moreover, few individuals were captured in BNP and BGB (Table 2). Total capture success was 18%; the results were highest at the ALF (p < 0.05) in September (p < 0.05), as shown in Figure 2.

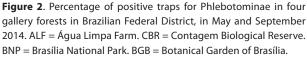
From the 472 phlebotomines captured at the ALF, 276 (58.5%) were identified as *Psathyromyia pradobarrientosi*, which was also the most frequent in May (90.3%). This species was followed by *Brumptomyia guimaraesi* and *Pa. bigeniculata*, both with 41 specimens

Curreita	ALF			CBR			BNP			BGB			T . 1	0/
Species	May	Sep	%	May	Sep	%	May	Sep	%	Мау	Sep	%	Total	%
Bichromomyia flaviscutellata	0	0	0	454	178	95.1	31	5	56.2	0	0	0	668	55.3
Brumptomyia brumpti	2	0	0.4	0	0	0	0	0	0	0	0	0	2	0.2
Brumpotomyia guimaraesi	0	29	6.1	0	0	0	0	0	0	0	2	22.2	31	3.6
Brumptomyia sp.	0	41	8.7	0	0	0	0	0	0	0	0	0	41	2.4
Evandromyia bourrouli	0	7	1.5	0	10	1.5	0	0	0	0	1	11.1	18	1.5
Mycropygomyia longipennis	1	2	0.6	0	7	1	0	0	0	0	0	0	10	0.8
Mycropygomyia ferreirana	0	0	0	0	4	0.6	0	0	0	0	0	0	4	0.3
Micropygomyia sp.	0	1	0.2	0	0	0	0	0	0	0	0	0	1	0.1
Nyssomyia whitmani	0	0	0	6	4	1.5	16	0	25	1	0	11.1	27	2.2
Psathyromyia abonnenci	1	0	0.2	0	0	0	5	0	7.8	0	0	0	6	0.5
Psathyromyia aragaoi	0	1	0.2	0	0	0	0	0	0	0	0	0	1	0.1
Psathyromyia bigeniculata	0	41	8.7	0	0	0	0	1	1.6	0	1	11.1	43	3.6
Pintomyia christenseni	0	26	5.5	0	0	0	0	0	0	0	0	0	26	2.2
Pintomyia kuscheli	0	1	0.2	0	0	0	0	0	0	0	0	0	1	0.1
Psathyromyia lutziana	10	7	3.6	0	0	0	1	0	1.6	1	0	11.1	19	1.3
Pintomyia monticola	1	22	4.9	0	0	0	0	0	0	0	0	0	23	1.9
Psathyromyia pradobarrientosi	100	176	58.5	1	0	0.2	5	0	7.8	1	2	33.3	285	23.6
Sciopemyia sordellii	1	2	0.6	0	0	0	0	0	0	0	0	0	3	0.2
Total	116	356		461	203		58	6		3	6		1.209	

Table 2. Phlebotomine species captured in the 4 areas studied in the Brazilian Federal District, in May and September 2014. Total species richness in the gallery forests: 16 species.

ALF = Água Limpa Farm. CBR= Contagem Biological Reserve. BNP = Brasília National Park. BGB = Botanical Garden of Brasília.





each (11.5%). These species were particularly abundant in September (Table 2).

A total of 664 specimens were collected in the CBR. From these, *Bichromomyia flaviscutellata* was the most abundant both in May (N=454; 98.4%) and September (N= 178; 87.7%). Low frequencies of *Nyssomyia whitmani* and *Evandromyia bourrouli* were observed (Table 2).

In BNP, a total of 64 phlebotomines were collected, 36 of which were *Bi. flaviscutellata* (56.25%). The second most common species in the BNP was *Ny. whitmani*, and overall, more species were collected in May than in September (Table 2). In the BGB, capture success was very low. Only 9 specimens were collected (Table 2).

This study found 5 new records: *Psathyromyia pradobarrientosi* is reported for the first time in Brazil and the Cerrado biome; *Brumptomyia guimaraesi*, *Br. brumpti*, *Mi. ferreirana*, and *Ev. bourrouli* are reported in the FD for the first time herein. In addition, 2 species known as vectors were collected in the gallery forests sampled: *Ny. whitmani* and *Bi. flaviscutellata*. The other species are not of any known epidemiological importance. This study has updated the list of sand fly fauna in the FD, where 35 species have now been recorded (Table 3).

Diagnoses of the newly recorded species.

Brumptomyia brumpti (Larrousse, 1920)

Material examined. Tables 1, 2; Figure 3a-c.

Male: superior proepimeral setae present; anepimeral setae absent; gonostyle with 2 external superior spines in the same tubercle; posterior spurs on the ascoids; ca 20 semi-foliaceous bristles on gonocoxite forming a tuft implanted directly on the structure (Fig. 3a); paramere not bifurcate and pilosous; aedeagus with conic format.

Brumptomyia guimaraesi (Coutinho & Barretto, 1941)

Material examined. Tables 1, 2; Figure 3a-c.

Male: superior proepimeral and anepimeral setae present (Fig. 3c); anepisternal inferior setae absent; gonostyle with 2 external superior spines on the same tubercle and presenting 2 apical spines; posterior spurs on ascoids; bristles on gonocoxite implanted in a compact and circular area (Fig. 3b); ejaculatory duct ca10× longer than ejaculatory pump.

Micropygomyia (Sauromyia) ferreirana (Barretto, Martins & Pellegrino, 1956)

Material examined. Tables 1, 2; Figure 4a-c.

Table 3 . Updated list of phlebotomine species in the Brazilian Federal District. Total species richness: 35 species.
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Family/ subfamily/tribe	SubTribe	Species	Habitat	Reference*
Psychodidae/	Brumptomyiina	Brumptomyia brumpti (Larrousse 1920)ª	Gallery forest	1
Phlebotominae/		Brumptomyia avellari (Costa Lima 1932)	_	3, 5
Phlebotomini		Brumptomyia pintoi (Costa Lima 1932)	_	3, 6
		Brumptomyia guimaraesi (Coutinho & Barretto 1941)ª	Gallery forest	1
	Sergentomyiina	Micropygomyia longipennis (Barretto 1946)	Gallery forest	1, 3, 5, 6
		Mycropygomyia ferreirana (Barretto, Martins & Pellegrino 1956) ^a	Gallery forest	1
		Mycropygomyia acanthopharynx (Martins, Falcão & Silva 1962)	_	3, 5, 6
		Mycropygomyia quinquefer (Dyar 1929)	Gallery forest	4
	Lutzomyiina	Evandromyia bourrouli (Barretto & Coutinho 1941)ª	Gallery forest	1
		Evandromyia bacula (Martins, Falcão & Silva 1965)	_	3, 5, 6
		Evandromyia teratodes (Martins, Falcão & Silva 1964)	_	3, 5, 6
		Evandromyia sallesi (Galvão & Coutinho 1939)	_	3, 5, 6
		Evandromyia corumbaensis (Galati, Nunes, Oshiro & Rego 1989)	_	3, 6
		Evandromyia saulensis (Floch & Abonnenc 1944)	_	3, 5, 6
		Evandromyia evandroi (Costa Lima & Antunes, 1936)	_	3, 5, 6
		Evandromyia lenti (Mangabeira 1938)	_	3, 5, 6
		Pintomyia christenseni (Young & Duncan 1994)	Gallery forest	1, 3, 6
		Pintomyia kuscheli (Le Pont, Martinez, Torrez-Espejo & Dujardin 1998)	Gallery forest	1, 4
		Pintomyia monticola (Costa Lima 1932)	Gallery forest	1, 4
		Pintomyia fischeri (Pinto 1926)	_	3, 6
		Lutzomyia longipalpis (Lutz & Neiva 1912)	Peridomicile	3, 5, 6
		Lutzomyia ischnacantha Martins, Souza & Falcão 1962	_	3, 6
		Sciopemyia servulolimai (Damasceno & Causey 1945)	_	3, 6
		Sciopemyia sordellii (Shannon & Del Ponte 1927)	Gallery forest	1, 3, 4, 5
		Psathyromyia lutziana (Costa Lima 1932)	Gallery forest	1, 3, 4, 5, 6
		Psathyromyia pradobarrientosi (Le Pont, Matias, Martinez & Dujardin 2004) ^{a,b, c}	Gallery forest	1
		Psathyromyia brasiliensis (Costa Lima 1932)	_	3,6
		Psathyromyia abonnenci (Floch & Chassignet 1947) ^d	Gallery forest	1, 4, 5
		Psathyromyia aragaoi (Costa Lima 1932)	Gallery forest	1, 3, 5, 6
		Psathyromyia bigeniculata (Floch & Abonnenc 1941) ^d	Gallery forest	1, 4, 5
	Psychodopygina	Psychodopygus davisi (Root 1934)	_	3
	, .,,	Nyssomyia whitmani (Antunes & Coutinho 1939)	Gallery forest, peridomicile	1, 3, 4, 5, 6
		Nyssomyia neivai (Pinto 1926)	_	3, 5, 6
		Nyssomyia intermedia (Lutz & Neiva 1912)	_	2, 3, 6
		Bichromomyia flaviscutellata (Mangabeira 1942)	Gallery forest, peridomicile	1, 3, 4, 5, 6

*References: 1 = current study; 2 = Carvalho et al. (1989); 3 = Carvalho et al. (2010); 4 = Ferreira et al. (2014); 5 = Almeida et al. (2015); 6 = Galati (2015). ^a First record in the Federal District.

^b Species erroneously identified as *Pa. runoides* (see details in the text).

^cFirst record in Brazil.

^d Species erroneously identified as *Pa. shannoni*, voucher numbers: *Psathyromyia pradobarrientosi* # ALFPap 1 – 276, CBRPap 1, BNPPap 1 – 5, BGBPap 1 – 3; *Brumptomyia guimaraesi* # ALFBrg 1 – 29, # BGBBrg 1 – 2; *Brumptomyia brumpti* # ALFBrb 1 – 2; *Micropygomyia ferreirana* # CBRMif 1 – 4; *Evandromyia bourrouli* # ALFEvb 1 – 7, CBREvb 1 – 10, BGBEv 1. - no data about specific habitat. ALF = Água Limpa Farm. CBR= Contagem Biological Reserve. BNP = Brasília National Park. BGB = Botanical Garden of Brasília.

Female: Segment II of the palpus shorter than IV (Fig. 4a); setae on the katepisternum and post-alar setae absent; labial fork present; pharynx with developed spines (Fig. 4b); 2 pairs of posterior teeth in the cibarium (Fig. 4b); absence of sclerotinized points in cibarial camara; fagel-lomere III with same length as labrum–epipharynx; body of the spermathecae with 2 distinct rings (Fig. 4c).

Evandromyia (Evandromyia) bourrouli (Barretto & Coutinho, 1941)

Material examined. Tables 1, 2; Figure 5a-c.

Female: eyes longer than clypeus (Fig. 5a); mesonotum and pleura brown; common duct of spermathecae reach-

ing middle of genital fork; body of spermathecae striate (Fig. 5b).

Male: gonocoxite with basal bristles (Fig. 5c); gonostyle with external spines isolated (Fig. 5c); lateral lobe with 3 spatulated setae (Fig. 5c); paramere not bifurcated.

Psathyromyia (Forattiniella) pradobarrientosi (Le Pont, Matias, Martinez & Dujardin, 2004)

Material examined. Tables 1, 2; Figure 6a-c.

Male: posterior spur on the ascoids (Fig. 6a); ejaculatory duct ca $3.2-5.7 \times$ longer than pump (Fig. 6b); gonostyle with external spine at its middle (Fig. 6b); ejaculatory

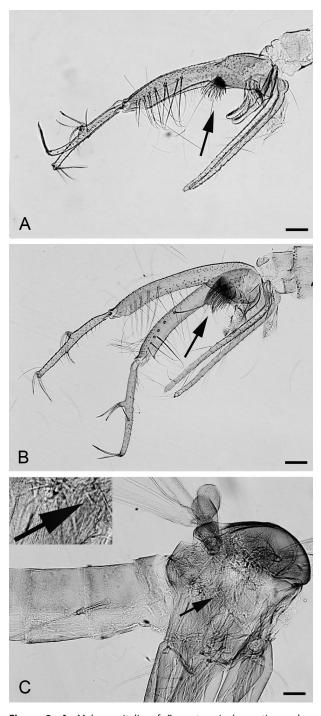


Figure 3. A. Male genitalia of *Brumptomyia brumpti*, voucher number ALFBrb 1; setae shows semi-foliaceous bristles on the gonocoxite forming a tuft implanted directly on the structure. **B.** Male genitalia of *B. guimaraesi*, voucher number ALFBrg 20; setae shows bristles on the gonocoxite implanted in a compact and circular area. **C.** Thorax of the male of *B. guimaraesi*; voucher number ALFBrg 20; setae shows superior proepimeral and anepimeral setae present; in the detail is the anepimeral region highlighted. Scale bars = 100 µm.

duct with losangular tip (Fig. 6c) and $2 \times$ the width of the anterior portion; aedeagus short.

Discussion

This study contributes to the knowledge of the phlebotomine fauna in the gallery forests of FD, a region which has been considered endemic for VL since 2005. Between 2010 and 2015, the number of species found in the FD increased to 35, 5 of which were recorded for the first time in the current study.

Because of urban expansion, deforestation has been the main factor affecting environmental changes in the FD. In the CBR, residences are located close to the remaining fragments of the Cerrado and even of the gallery forests. As a result, there is interaction between wild fauna and humans, as well as between these sylvatic species and domestic animals. These confined animals become a new, easy, and safe blood source for sand flies. The finding of species of epidemiological importance in the gallery forest of the BNP (and the presence of Ny. whitmani in particular) means that extra attention is required in the completion of the urban development project involving the creation of a new neighborhood in the northwestern sector of the FD. The ecological impact of human activity on the area may be reflected in the increased number of CL cases in dogs and humans in the area (Cardoso et al. 2015).

The main neighborhoods that share borders with the BGB, which are known as Lago Sul and Jardim Botânico, also experience strong human pressure due to overcrowding and urbanization in the Cerrado that surrounds the region. The occurrence of phlebotomine sand flies in BGP represents a risk of the possible expansion of leishmaniasis to humans and domesticated animals in the region.

The ALF exhibited the greatest species richness. Among the species found in the ALF, Pa. pradobarrientosi was the most frequently captured. This species was found in the other 3 study areas as well. Pa. pradobarrientosi was also the most abundant species in the BGB. The male of Pa. pradobarrientosi had been described previously from the Bolivian Amazon during the rainy season. The occurrence of this species in Brazilian FD (in all areas sampled in the current study) may be associated with the presence of forest connections between Brazilian Cerrado and Bolivian Amazon. The gallery forests may function as a refuge for many phlebotomine species and as ecological corridors in which these insects move along the riverbanks, as has been suggested in studies on other faunal groups (Moraes et al. 2015). It is a species morphologically similar to Pa. runoides, so it was long confused with it (Le Pont et al. 2004). Ferreira et al. (2015) recorded Pa. runoides in FD, but after reviewing the slides, one of us (AJA), based in Galati's taxonomic proposal observed a misidentification. The description of the female is still being developed (AJA, personal communication).

Sábio et al. (2014, 2016a, b) reviewed the taxonomic status of the species within the subgenus *Psathyromyia* Barretto, 1962 showing that *Pa. shannoni* does not occur in Brazil. The species *Pa. shannoni*, *Pa. abonnenci*, *Pa. limai*, *Pa. bigeniculata*, *Pa. pifanoi*, *Pa. baratai* and *Pa. ribeirensis* are still included in a taxonomic category named shannoni series (Sábio et al. 2016b) due the aspect of the spermathecae of the females. Ferreira et al. (2015) recorded *Pa. shannoni* in the FD, but based in the

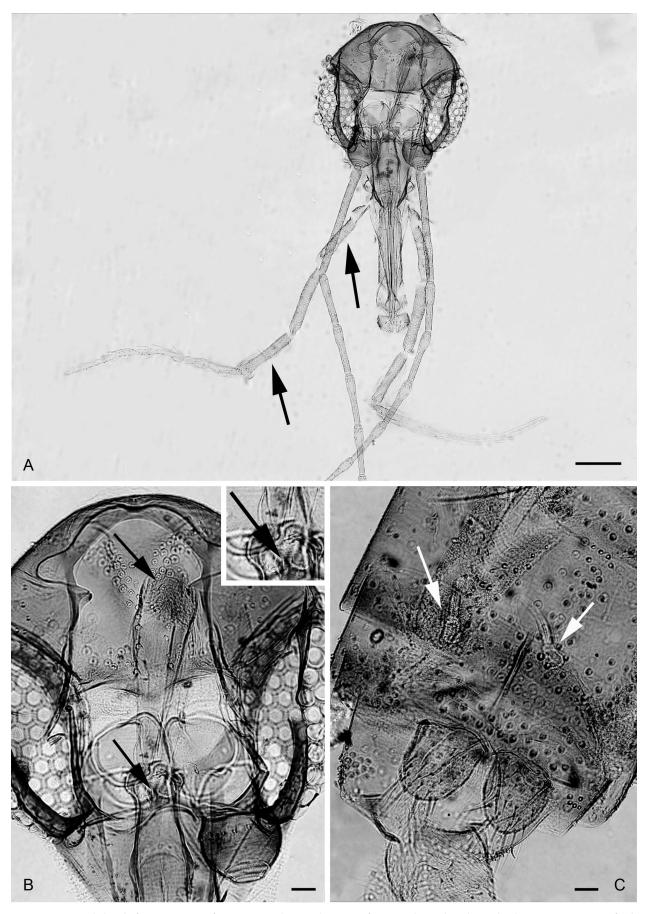


Figure 4. A. Female head of *Micropygomyia ferreirana*, voucher number CBRMif 1; setae shows the relationship between II segment of palpus (basal setae) and IV segment of palpus (proximal setae). **B.** Pharynx and cibarium of the female of *M. ferreirana* voucher number RM247; setae shows the spines in the pharynx and the teeth of cibarium; in the detail cibarium is highlighted. **C.** Spermathecae of *M. ferreirana*, voucher number CBRMif 1; setae shows the head of the structure. Scale bars: $A = 100 \mu m$; $B, C = 20 \mu m$.

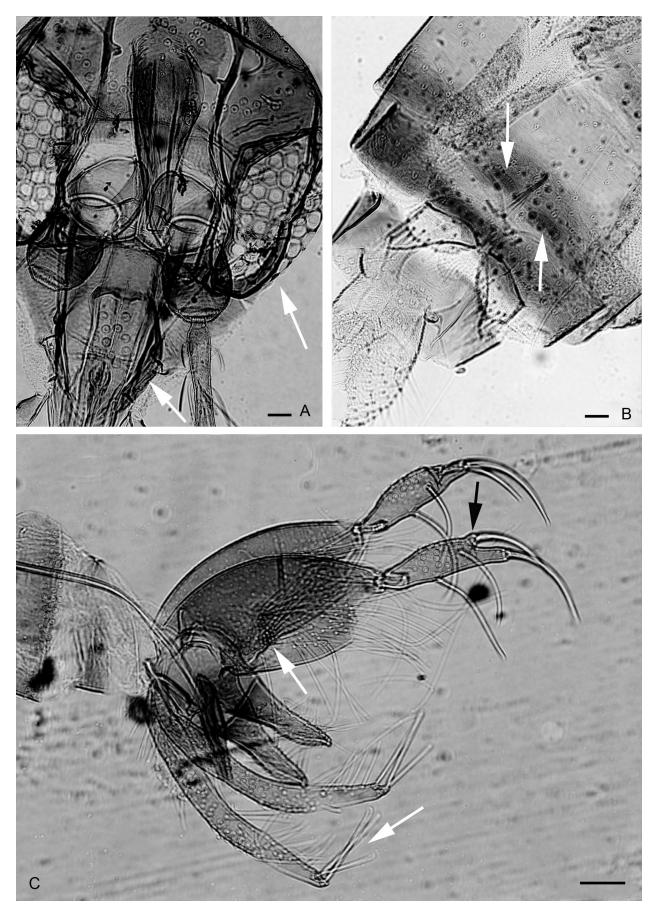


Figure 5. **A.** Pharynx and cibarium of the female of *Evandromyia bourrouli*, voucher number ALFEvb 1; setae shows the relationship between the eye and the clypeus. **B.** Spermathecae of *E. bourrouli*, voucher number ALFEvb 2; setae shows the body of the structure. **C.** Male genitalia of *E. bourrouli*, voucher number ALFEvb 7; setae shows the bristles of the gonocoxite and the lateral lobe with three spatulated setae. Scale bars: A, B = 20 μ m; C = 50 μ m.

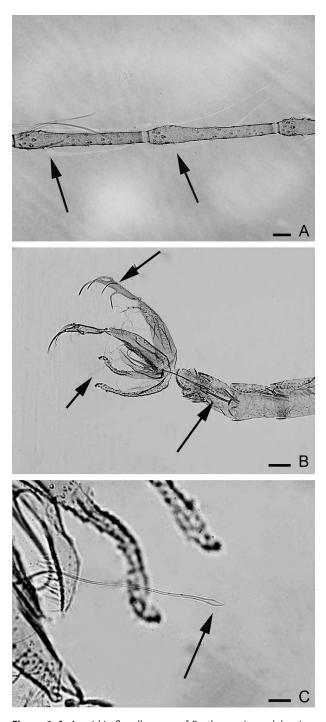


Figure 6. A. Ascoid in flagellomeres of *Psathyromyia pradobarrientosi*, voucher number BNPPap 1; setae shows the ascoids. **B.** Male genitalia of *P. pradobarrientosi*, voucher number ALFPap 1; setae shows the relationship between the pump and the ejaculatory duct and the external spine in the middle of the gonostyle. **C.** Tip of the ejaculatory duct of *P. pradobarrientosi*, voucher number ALFPap 1; setae shows the losangular format of the structure. Scale bars: A, B = 20 μ m; C = 100 μ m.

key published by Sábio et al. (2014) and Galati (2015) it was possible to revise the identification of the species previously identified as *Pa. shannoni*. *Psathyromyia bigeniculata* was a junior synonym of *Pa. shannoni* that was revalidated by Sábio et al. (2014) and correctly identified in the present study. Specimens of *Pa. bigeniculata* can be distinguished of *Pa. abonnenci* based in the thoracic coloration and males can be also separated using the distribution of the bristles on the dorsal margin of the paramere.

Brumptomyia guimaraesi specimens were collected in the ALF and the BGB, while *Brumptomyia brumpti* was only collected in the ALF. *Brumptomyia* species are not important in the eco-epidemiology of leishmaniasis (Eckert and Souza 2010), and according to Aguiar and Medeiros (2003), these species suck blood from armadillos (Edentata, Dasypodidae). The insects are always found on fallen leaves on the forest floor and in mammal dens (Eckert and Souza 2010, Cutolo et al. 2013).

Four *Mycropygomyia ferreirana* specimens were found in the CBR in September and in the Center-West Region of Brazil was recorded only in Mato Grosso do Sul (Galati 2015). Some species of the genus *Micropygomyia*, subgenus *Sauromyia*, such as *Mi. quinquefer* (Paiva et al. 2010), *Mi. ferreirana* (Rocha et al. 2010, Rêgo et al. 2015), *Mi. capixaba*, *Mi. villelai*, and *Mi. peresi* (Rêgo et al. 2015) have been recorded infected by *Leishmania* species through molecular analyzes in some states of Brazil. According to Aguiar and Medeiros (2003), species included in these genera bite cold-blooded animals, but as mentioned by Rêgo et al. (2015) the finding of *Leishmania* DNA in this genus suggests that these sand flies have fed on hosts susceptible to parasite infection.

Evandromyia bourrouli, a species inhabiting forests and armadillo dens (Dorval et al. 2010), was captured only in September. Dorval et al. (2010) found a high density of this species in the state of Mato Grosso do Sul using Disney traps. According to their study, almost all females were found engorged, which seemed to reflect their affinity for rodents as a source of blood. They also noted that this species has been collected in forest areas and human environments, particularly those in the Center-West and Northeastern regions of Brazil. Araújo-Pereira et al. (2014) found Ev. bourrouli in forests in the Brazilian state of Acre, and Vilela et al. (2013) reported this species in rural and periurban areas in Guaraí (Tocantins state). Evandromyia bourrouli has been found in many different habitats ranging from preserved forests to animal shelters (chicken coops and pigpens), which reflects their eclectic behavior. However, this species has not been reported as a vector of pathogens to humans.

The predominance of *Bi. flaviscutellata* was expected in forested areas as reported by Carvalho et al. (2010), who compared sand flies captures in households, peridomestic environments, and gallery forests. The current study found many specimens of *Bi. flaviscutellata*, particularly in the CBR and the BNP. Given the presence of small mammals infected by *Leishmania* (*Leishmania*) *amazonensis* in these areas (Cardoso et al. 2015), this species may be responsible for the enzootic circulation of this parasite, which is an etiological agent of diffuse cutaneous leishmaniasis.

Similarly, *Ny. whitmani* was captured in the CBR and the BNP, as well as in the BGB. Carvalho et al. (2010) found the species in all the locations and habitats studied in the FD (households, peridomestic environments, and gallery forests). This finding is indicative of the potential degree of domiciliation of this species. It reflects the importance of this species as a *Leishmania braziliensis* vector in the FD. *Ny. whitmani* is also considered the most important CL vector in many parts of Brazil (Costa et al. 2007).

Psathyromyia pradobarrientosi, Pa. lutziana, Mi. longipennis, Sciopemvia sordellii, and Pintomvia monticola were found in the ALF in May and September. In both months, Pa. pradobarrientosi was found in the BGB, Bi. flaviscutellata and Ny. whitmani were found in the CBR, and Bi. flaviscutellata was found in the BNP. The other species were found to be distributed irregularly between these 2 months. These monthly differences in abundance and occurrence indicate that these species respond differently depending on the presence of different blood-based food sources, breeding grounds, shelters, and the season. However, the small area of influence of light traps is important; the attraction radius for phlebotomines (small insects) is not more than 5 m (Dye et al. 1991). The success of samplings, therefore, depends on the displacement of species within the geographical space studied (where the traps are distributed).

No *Lu. longipalpis* specimens were captured in the gallery forests studied. This species is currently listed as the main VL vector in the Americas. Initially, VL was associated with rural habitats and the outskirts of large cities; however, this profile has changed in certain regions where the disease has been found to be urbanized. This process has been associated with environmental modifications caused by human activity, by the rapid migratory process, by the interactions between wild reservoirs and infected dogs and their mobilization into areas without transmission, and by the adaptation of the vector *Lu. longipalpis* to peridomestic environments (Vilela et al. 2013).

Changes in behavior exhibited by *Lu. longipalpis* enable this species to adapt to increasing losses in biodiversity. Moreover, the frequent exposure to insecticides in urban areas rendered them resistant. These changes may explain population increases of this species and the consequent propagation of the disease to peridomestic and domestic habitats (Aguiar et al. 2014, Salomón et al. 2015). The absence of this species in the gallery forests sampled reflects its adaptive nature, which may be reflected in its apparently opportunistic behavior in relation to sources of carbohydrates and blood (Brazil 2013). This is especially likely, given its presence in peridomestic and domestic habitats in the FD (Carvalho et al. 2010). From an evolutionary perspective, this behavior may have favored its vector competence in urban areas (Brazil 2013).

Little is known about the sand fly fauna in the FD relative to other regions in Brazil. Carvalho et al. (2010) listed 27 phlebotomine species in different habitats in the FD. The current study has found 35 species in FD. This number of species is representative of the region, given the fact that 47 species were reported in Goiás, which is approximately 59 times the size of the FD (Almeida et al. 2015). Future studies with efforts focused on sampling

methods similar to those of the current research and on other vegetation types may be able to reveal a larger species richness in the FD. Relevant information on species richness, abundance, and occurrence at different periods of the year may be used to better understand the epidemiology of diseases and to increase knowledge available on phlebotomine behavior in the gallery forests close to the urban areas of the FD.

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Authors' Contributions

AR collected the data, processed the material, and wrote the text and tables. AJA collected the data, identified the specimens, wrote the diagnoses, and coordinated the work. DAR collected the data and photographed the material. JCBF collected the data. RVT processed the material. MTO collected the data and RGG collected and analyzed the data, prepared the figures, and coordinated the work. All authors reviewed the manuscript.

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