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Lutzomyia wellcomei Fraiha, Shaw & Lainson (Diptera, Psychodidae, Phlebotominae) in an Atlantic Forest Remanant of Rio Grande do Norte, Northeastern Brazil

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Abstract. Leishmaniasis can affect humans and animals in visceral or cutaneous forms. The cutaneous form has been associated, among other species, with *Lutzomyia wellcomei* Fraiha, Shaw & Lainson, initially found in the Amazon region. The present study is based on monthly collections from January to December in an Atlantic Forest remnant and a peridomestic environment, using CDC light traps. This is the first systematized study of the specie in Rio Grande do Norte State. The insects were metalized under carbon tape, visualized and photographed with a scanning electron microscope. The most abundant species in the forest area was *Lutzomyia walkeri* Newstead followed by *L. wellcomei*. Of the total captured in the forest and the peridomestic environment, around 8% were *L. wellcomei*, the predominant species in the forest environment in the rainy season. *L. wellcomei* sandflies were not captured in the peridomestic environment. In the forest this species was found along with *Lutzomyia whitmani* Antunes & Coutinho and *Lutzomyia intermedia* Lutz & Neiva. Male and female *Lutzomyia longipalpis* Lutz & Neiva were predominant in the peridomestic environment. Microscopic analysis of the external morphology of *L. wellcomei* reveals details of male and female heads.

Keywords: Leishmaniasis; Sandflies; Vector.

Lutzomyia wellcomei Fraiha, Shaw & Lainson (Diptera, Psychodidae, Phlebotominae) em Fragmento de Mata Atlântica do Rio Grande do Norte, Nordeste do Brasil

Resumo. As leishmanioses podem afetar seres humanos e animais nas formas visceral e tegumentar. A forma tegumentar tem sido associada, entre outras espécies, a *Lutzomyia wellcomei* Fraiha, Shaw & Lainson, inicialmente encontrado na região amazônica. O estudo se baseou em coletas mensais de janeiro a dezembro em um fragmento de Mata Atlântica e em um ambiente peridomiciliar, com armadilhas luminosas CDC. Esse é o primeiro estudo sistematizado sobre essa espécie no Rio Grande do Norte. Os insetos foram identificados e analisados em relação às chuvas no local. Alguns espécimes de *L. wellcomei* foram metalizados sob fita de carbono, visualizados e fotografados em microscópio eletrônico de varredura. A espécie mais abundante na área de mata foi *Lutzomyia walkeri* Newstead seguida por *L. wellcomei*. Do total capturado no ambiente de mata e no peridomicílio, cerca de 8% corresponde a *L. wellcomei*, sendo esta a espécie predominante no ambiente de mata no período chuvoso. Flebotomíneos *L. wellcomei* não foram capturados em ambiente peridomiciliar. Na mata essa espécie foi encontrada juntamente às espécies *Lutzomyia whitmani* Antunes & Coutinho e *Lutzomyia intermedia* Lutz & Neiva. Machos e fêmeas *Lutzomyia longipalpis* Lutz & Neiva foram predominantes no ambiente peridomiciliar. A análise microscópica da morfologia externa de *L. wellcomei* revela detalhes da cabeça de machos e fêmeas.

Palavras-Chave: Leishmanioses; Flebotomíneos; Vetor.

Leishmaniases are widely distributed diseases caused by protozoa of the genus *Leishmania*. Depending on the species, it can present itself in the visceral or cutaneous form, affecting humans and animals. Cutaneous Leishmaniasis (CL), as well as Visceral Leishmaniasis (VL), generally occur in areas with precarious socioeconomic conditions, reflecting a serious public health problem that requires increasing understanding of the various socio-environmental aspects involved in disseminating the disease in Old and New World countries. International organizations are currently analyzing the recent epidemic outbreak of CL, caused by *Leishmania tropica* Wright and transmitted by *Phlebotomus papatasi* Scopoli, in a war-torn area of Syria (Marzochi &Marzochi 1994; Scott 2005; Alasaad 2013).

The most frequent clinical manifestation is the cutaneous form, caused primarily by *Leishmania major* Yakimoff & Schokhor and *L. tropica* in the Old World, and by *Leishmania braziliensis* Vianna and *Leishmania mexicana* Biagi in the New World (Berman 1997; Reithinger *et al.* 2007), with 90% of cases in Afghanistan, Algeria, Iran, Saudi Arabia, Syria, Brazil, Colombia, Peru and Bolivia (Desjeux 2004; Modabber *et al.* 2007).

Studies indicate that climatic changes are important in the geographic expansion of vectors and, consequently, in the transmission of several forms of the disease and that deforestation contributes to the increased number of cases in periurban and urban areas. Isolated cases or epidemic outbreaks have been reported when humans occupy forest environments for economic or military reasons, or when their dwellings are constructed near forest areas (Patz *et al.* 2000;Campbell-Lendrum *et al.* 2001; Bern *et al.* 2008).

In the Americas, cases of cutaneous leishmaniasis occur from the United States to Argentina, with Brazil exhibiting the highest incidence on the continent (Rabello *et al.* 2003; Reithinger *et al.* 2007; Llanos-Cuentas *et al.* 2008; Negrão & Ferreira 2009). Amazonia has the greatest diversity of disease-causing parasites,

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the primary species being *Leishmania amazonensis* Lainson & Shaw, *L. braziliensis* and *Leishmania guyanensis* Floch (Coutinho *et al.* 1987).

The main vector species of the different forms of ACL in Brazil are *Lutzomyia intermedia* Lutz & Neiva (Rangel *et al.* 1986, 1990, 1992), *Lutzomyia migonei* França (Pessôa & Coutinho 1941; Queiroz *et al.* 1994), *Lutzomyia whitmani* Antunes & Coutinho (Azevedo *et al.* 1990; Alessi *et al.* 2009), *Lutzomyia umbratilis* Ward & Fraiha (Cabanillas & Castellón 1999), *Lutzomyia wellcomei* Fraiha, Shaw & Lainson (Ready *et al.* 1983) and *Lutzomyia complexa* Mangabeira (Lainson & Shaw 1998). These species account for the transmission of *L. braziliensis* in different regions of Brazil (Lainson *et al.* 1973; Rangel *et al.* 1992; Queiroz *et al.* 1994; Lainson & Shaw 1998), except *L. whitmani*, which also transmit *Leishmania shawi* Lainson, Braga, de Souza, Póvoa, Ishikawa & Silveira (Shaw *et al.* 1991), and *L. umbratilis*, incriminated as vetors of *L. guyanensis* (Lainson 1983), both in Amazonia.

Despite the variety of *Lutzomyia* species possibly involved in transmission, there is a clear dependency on interactions between vectors, host animals and the species of *Leishmania*, that is, the natural infection of these species by the parasite, degree of anthropophilism of the vectors, occurrence of cases of the disease and interaction with local abiotic factors.

L. wellcomei, a vector of *L. braziliensis*, especially in mountainous regions (Ready *et al.* 1983), was initially described only in the Amazon region (Lainson *et al.* 1973; Lainson & Shaw 1979), and later in forest environments of Ceará state (Ready *et al.* 1983), in forest and peridomestic areas on São Luis Island in Maranhão state (Rebêlo *et al.* 1999) and more recently, in a wooded area of a metropolitan region of Pernambuco (Silva & Vasconcelos 2005) and in Rio Grande do Norte (Cortez 2006; Pinheiro 2010).

Despite the crepuscular and nocturnal habits of most sandflies, *L. wellcomei* was collected while biting a man during the daytime. A high degree of anthropophilism was also recorded (Fraiha *et al.* 1971; Ward *et al.* 1973).

The description of the morphological characteristics of sandflies has contributed to understanding the taxonomy and distribution of new species of the genus *Lutzomyia* (Bejarano *et al.* 2004).

The incidence of ACL has been increasing in almost all the Brazilian states, with outbreaks in the Southeast, Midwest and Northeast. In Rio Grande do Norte, cases of the disease occur primarily in the Apodi region. An earlier study demonstrated that the disease is caused by *L. braziliensis* (Oliveira *et al.* 2004). Despite the occurrence of the disease and the recent increase in the number of cases, infection caused by *L. braziliensis* has yet to be identified, a fact that justifies this and other studies.

MATERIAL AND METHODS

Study Site. The study was conducted in an Atlantic Forest fragment, and a peridomestic setting, located in the Rommel Mesquita de Faria Experimental Station (05º 56' S and 35º 11' W), belonging to the Agricultural Research Corporation of Rio Grande do Norte (EMPARN), in the township of Parnamirim, in the metropolitan region of Natal (Figure 1).

The preserved Atlantic Forest remnant, known as Mata do Jiqui, encompasses 79 ha, with central coordinates 5º56' S and 35º11' W. Vegetation has a density of 1526 live trees per hectare. The 59 arboreal species found in the area are distributed among 31 families, the Leguminosae and Myrtaceae being the most representative with 11 and 10 species, respectively, followed by Bignoniaceae and Rubiaceae, with three species each (Cestaro & Soares 2008).

The peridomestic environment consists of a set of dwellings occupied by station employees. In this area plant mass is composed primarily of herbaceous vegetation and fruit trees such as the jackfruit (*Artocarpus heterophyllus* Lam.), guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), orange (*Citrus sinensis* L.) and acerola (*Malpighia glabra* L.). Sheep

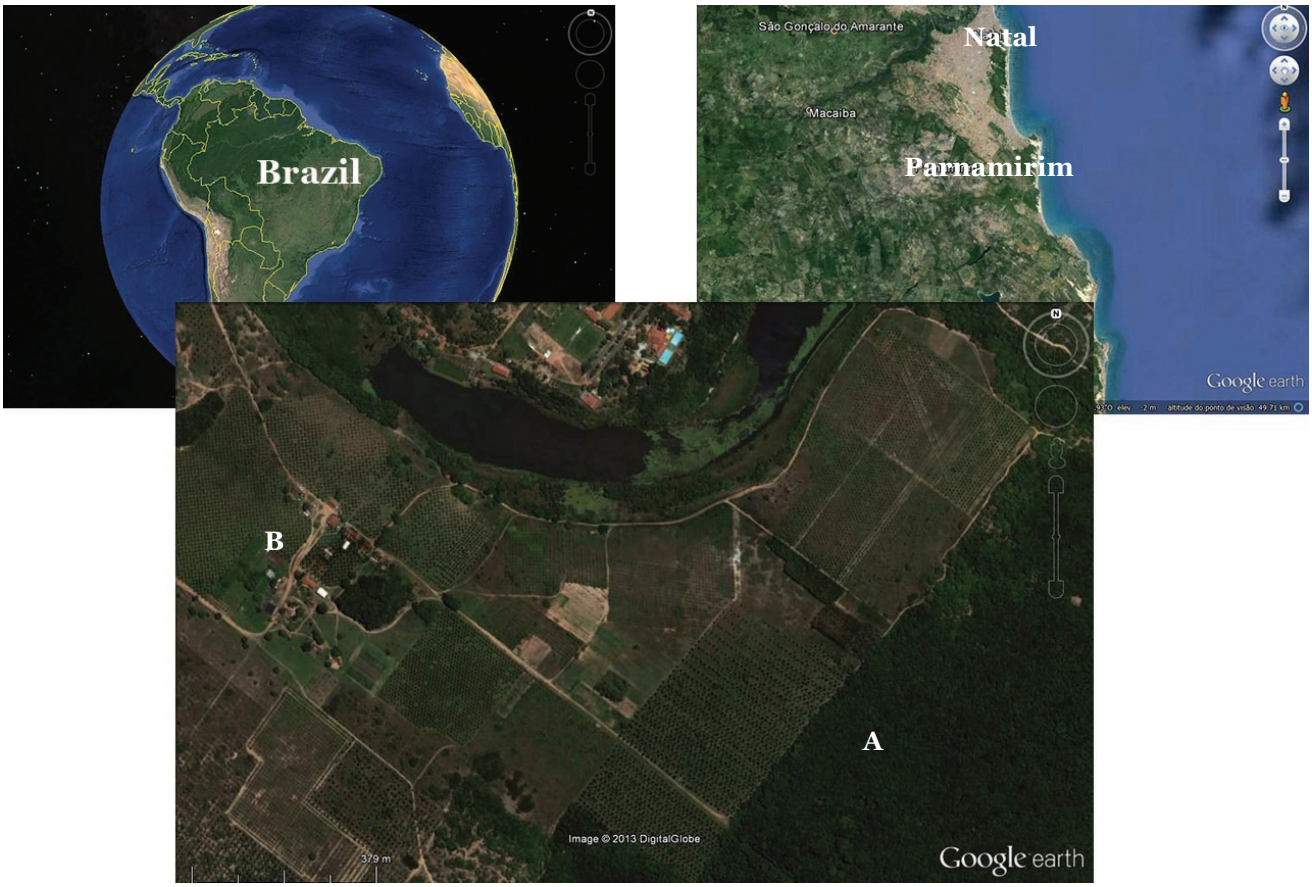

Figure 1. Collection area indicating the forest (A) and peridomestic environment (B).

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(*Ovis aries* L.), dogs (*Canis familiaris* L.) and chickens (*Gallus domesticus* L.) also inhabit the area.

Capture and sandly identification. Collections took place between January and December, with CDC light traps installed 1 meter from the ground at 5 pm and removed at 7 am the following day, on 3 consecutive days each month. Rainfall data were obtained at the collection site.

The trap in the forest remnant was installed in a tree, approximately 100 meters from the edge. In the peridomestic environment, it was placed between a chicken coop and the bedroom of one of the employees.

The traps were removed in the morning and the cages containing the insects were taken to the Entomology Laboratory (LabEnt) of the Federal University of Rio Grande do Norte. The insects were killed by freezing at -20ºC for 20 minutes, sandflies were sorted from the others, clarified in a solution of 10% potassium hydroxide (KOH), mounted and identified using Young & Duncan's (1994) taxonomic key.

Some specimens of *L. wellcomei* were metalized under carbon tape (BalTec SCD-005 metalizer), visualized and photographed with a scanning electron microscope (Phillips XL-30ESEM). Scanning electron microscopy micrographs were taken at the Scanning Electron Microscopy Laboratory of the Federal University of Rio Grande do Norte.

Statistical analysis. The analysis of diversity between the environments was obtained using the Shannon-Wiener Diversity Index (H') (Magurran 1988). To calculate this index, the Ecological Methodology software version 5.2 (Kenney & Krebs 2000) was used.

The Index of Species Abundance (ISA) and Standardized Index of Species Abundance (SISA) (Roberts & His 1979) were used to analyze the data obtained in Atlantic Forest and peridomestic environments. The ISA values were determined and converted in SISA to values between 0-1, using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

A total of 929 sandflies were collected, 92.7% in the Atlantic Forest remnant and the remainder in the peridomestic environnment (Table 1). The most abundant species was *Lutzomyia walkeri* Newstead, with 75% of individuals, followed by *L. wellcomei*, with7.8% and *Lutzomyia longipalpis* Lutz & Neiva with 7.1%.

In the forest area 555 males and 307 females from 8 species were collected, as follows: *L. walkeri, L. wellcomei, L. evandroi, L. whitmani, L. sordellii, L. longipalpis, L. intermedia and L. shannoni. L. walkeri* was the most abundant species, with 699 individuals, followed by *L. wellcomei* with 73 and *L. evandroi* with 52. *L. wellcomei* occurred only in the forest area during the rainy season, from April to September (Figure 2). *L. wellcomei*, *L whitmani* and *L. intermedia*, the three species involved in transmitting the etiologic agent of cutaneous leishmaniasis, were captured only in the forest environment, while *L. longipalpis*, a vector of *Leishmania chagasi* Chagas & Cunha, was predominant in the peridomestic (Table 1).

The Atlantic Forest area exhibited greater species diversity (H'=1.02) in this site, where all eight species were collected (Table 1). The species that occurred only in the forest area were *L. wellcomei*, *L. whitmani*, *L. sordellii*, *L. intermedia* and *L. shannoni*.

Only 3 species were captured in the peridomestic environment (H'=0,55), 51 males and 16 females. *L. longipalpis* was the most abundant species in this environment, followed by *L. evandroi* and *L. walkeri*, all with a decreasing tendency in the rainy season.

The most abundant species were *L. walkeri* with a SISA of 0.75, *L. evandroi* with 0.70 and *L. longipalpis* with 0.64, followed by *L. wellcomei* (0.25) and the others (Figure 3).

Analysis of the emergence of vector species in the two environments throughout the year (Figure 4), shows that *L. longipalpis* and *L. whitmani* exhibit peak occurrence in February, while for *L. wellcomei* it occurs in July.

Microscopic analysis (Figure 5) of the external morphology of *L. wellcomei* reveals details of male and female heads. The head (Figure 5B) shows large compound eyes of similar size and appearance; corneas clearly visualized; antennae long, palpi with five segments and piercing mouthparts. Females have a mandible and maxilla with pointed structures (Figures 5E and 5G). Figure 5F illustrates three of the five palpus segments and the lips. The hypopharynx contains teeth and a long food canal in the central part (Figure 5H). Three trichoid sensilla are found on the thoracic segment and in close proximity to the spiraculum, in addition to many small bristles (Figure 5D). Comparative studies, using electronic microscopy, analyze morphological differences between populations of a same species and their role as vectors in different areas. Morphological characteristics and genetic diversity were analyzed in *L. longipalpis*, vector of visceral leishmaniasis in Venezuela (Arrivilaga *et al.* 2000). Both males and females of *L. migonei* have mandibles, which are smaller in the former. Lateral teeth are reduced or absent. Only

Table 1. Sandflies collected in a peridomestic and forest environment, located in the Parnamirim, in the metropolitan region of Natal, between January and December.

Specie	Forest		Peridomestic		Total	%
	♂	♀	♂	♀		
Lutzomyia walkeri	525	174	1	0	700	75.3%
Lutzomyia wellcomei	11	62	0	0	73	7.8%
Lutzomyia longipalpis	0	7	46	13	66	7.1%
Lutzomyia evandroi	5	47	4	2	58	6.2%
Lutzomyia whitmani	12	0	0	0	12	1.3%
Lutzomyia sordellii	1	8	0	0	9	1%
Lutzomyia intermedia	1	0	0	0	1	0.1%
Lutzomyia shannoni	0	1	0	0	1	0.1%
Lutzomyia sp.	0	8	0	1	9	1%
Total	**555**	**307**	**51**	**16**	**929**	**100%**
	862		**67**			

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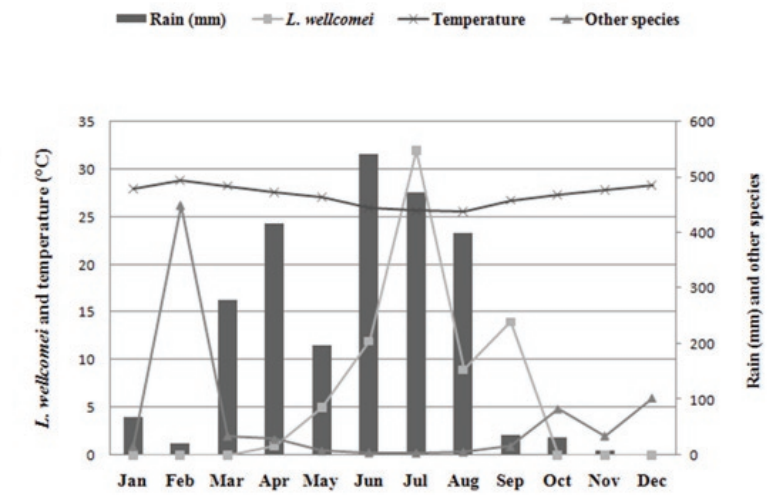

Figure 2. Annual distribution of *L. wellcomei* and rainfall.

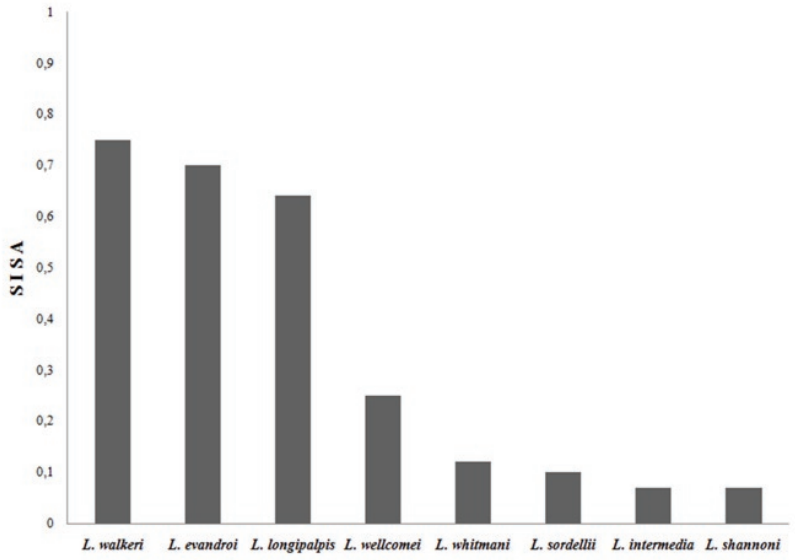

Figure 3. Results of Standardized Index of Species Abundance (SISA).

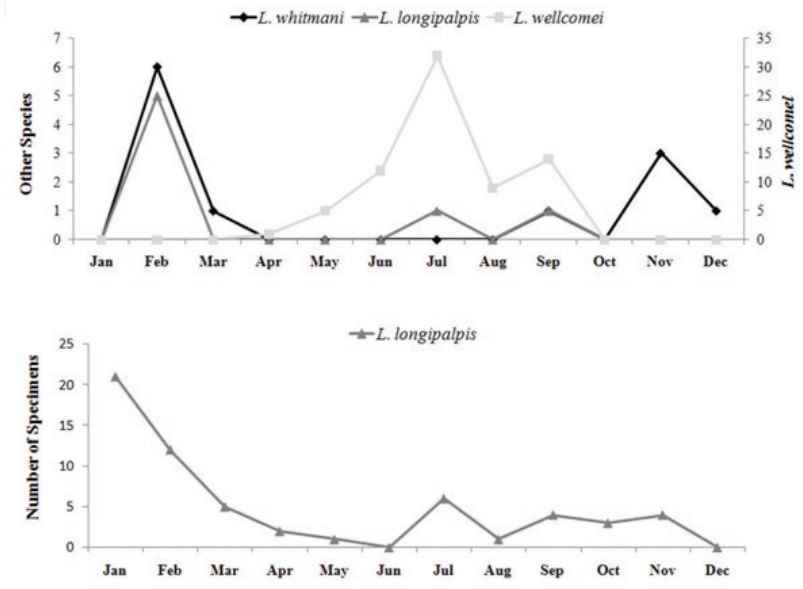

Figure 4: Annual distribution of potential vectors in Forest and Peridomestic environments.

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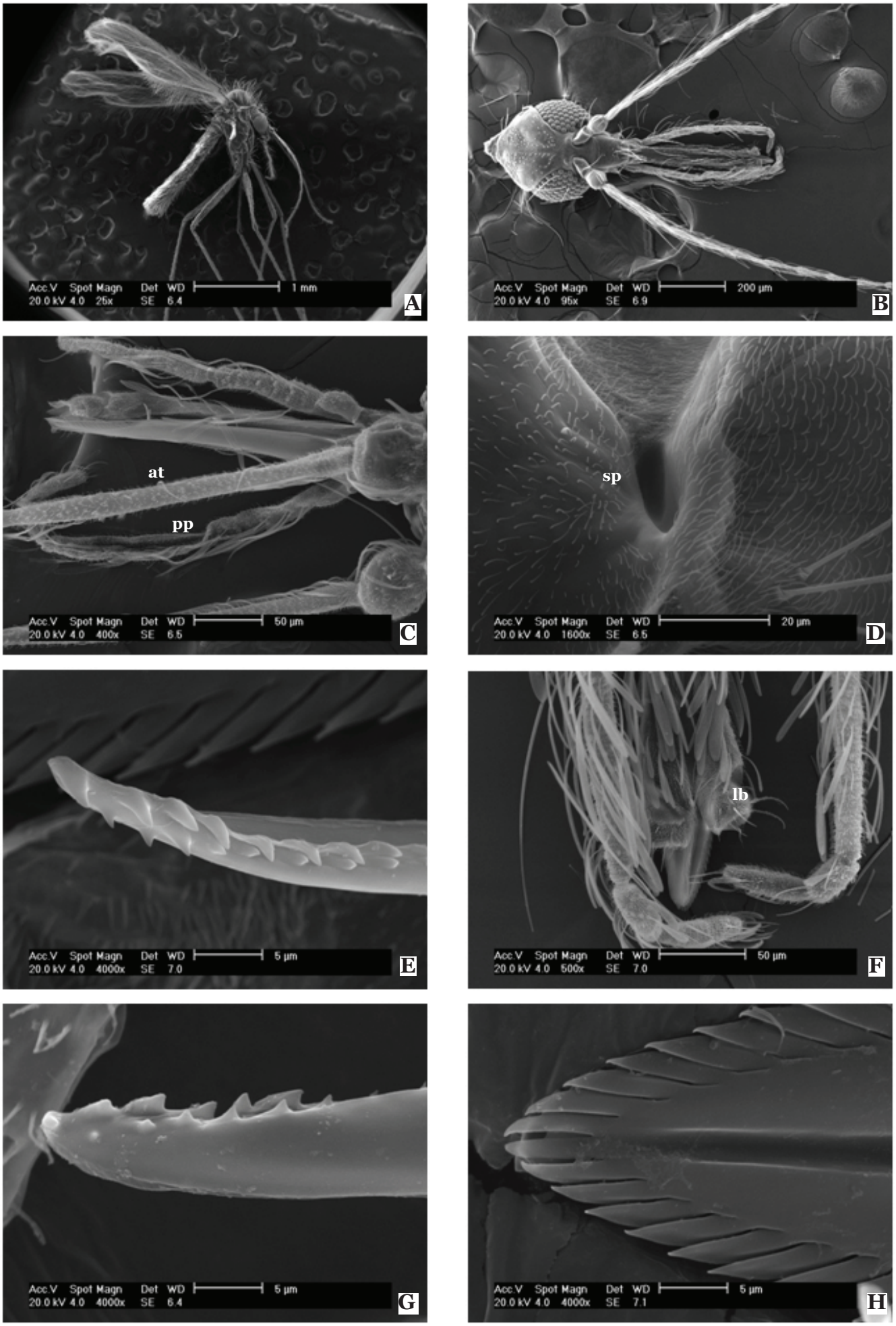

Figure 5. Scanning electron microscope micrographs of L. wellcomei: entire female – A; female head – B; male mouth, antennae (at) and palpi (pp) – C; female respiratory spiraculum (sp) – D; female maxilla (mx) – E, female palpi (pp) and lip (lp) – F; female mandible– G; female hypopharynx, in the center of the food canal – H.

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females have teeth at the tip of the hypopharyx, while in males they are substituted by spicules (Silva & Grunewald 2000).

Earlier studies have analyzed species diversity in Rio Grande do Norte, (Ximenes *et al.* 1999; Ximenes *et al.* 2000; Ximenes *et al.* 2007), but this is the first systematic investigation regarding the occurrence of *L. wellcomei* for the state. This species is a proven vector of *L. braziliensis,* the agent responsible for American cutaneous leishmaniasis (Lainson & Shaw 1979; Ryan *et al.* 1987).

In this study, *L. wellcomei* was not captured in the peridomestic environment, the site of the greatest occurrence of other species in the dry season, particularly *L. longipalpis*, the vector of visceral leishmaniasis, followed by *L. evandroi* (Table 1).

There is therefore a clear tendency towards the occurrence of *L. wellcomei* in the rainy season, as well as its behavior typical of a wild species, as shown by Lainson & Shaw (1998) in Amazonia. According to Fraiha *et al.* (1971), *L. wellcomei* exhibits intense anthrophilism, even during the day, a fact that justifies new analyses. Individuals in the study area work in close proximity to the forest. Moreover, the growing urban expansion has resulted in several housing developments in areas bordering the forest, which may favor the occurrence of cases in this region, as observed in other parts of Brazil and in other countries (Bern *et al.* 2008).

The occurrence of these insects in forest areas of the Northeast (Ready *et al.* 1983; Rebêlo *et al.* 1999; Silva & Vasconcelos 2005) may indicate their geographic expansion, or even their likely preexistence, as part of Atlantic Forest fauna, yet only recently discovered. Climatic changes and anthropic action, in the form of deforestation and construction in or near forest areas, may be related to the geographic expansion of sandfly species such as *L. wellcomei* and *L. whitmani* and consequently notification of cases in periurban areas. Thus, the results obtained here widen the knowledge base of the distribution and occurrence of cutaneous leishmaniasis vectors in Northeastern Brazil.

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