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# *Lutzomyia longipalpis* Breeding—A Probable Breeding Substrate for *Lutzomyia longipalpis* in Nature

**João Carlos Araujo Carreira, Reginaldo Peçanha Brazil, Bianca dos Santos Carvalho, Alba Valéria Machado da Silva**

Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil
Email: jcacarreira@gmail.com

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## Abstract

The identifying of the preferred breeding sites of sand fly immature forms is quite important to the understanding of the vector's biology and the development of more effective control strategies for leishmaniasis. In the present study, it was analyzed experimentally; the potential of dog feces of serving as breeding substrate to *Lutzomyia longipalpis*. Two situations were tested: 1) the life cycle of the insects from larvae to adults, 2) the whole cycle from adults to adults. Our results demonstrated for the first time that: 1) *L. longipalpis* can be easily bred in dog feces, suggesting that it could represent a potential raising substrate to immature forms for this species; 2) *L. longipalpis* larvae are able to make galleries on a substrate, literally burying themselves in this to function also as a shelter; 3) from the previous information that dogs infected with *Ancylostoma caninum* can present dodecanoic acid in their feces, we suggested that this substrate may be particularly more attractive to *L. longipalpis* females seen that this molecule is the same component of their oviposition pheromone, which is produced by the accessory glands and secreted onto the eggs; 4) after the copulation the males may landing on the substrate and continued beating the wings intermittently until the death in a similar behavior also noticed in other situations associated to kairomones response or pheromone production. Therefore, we suggested that it is very likely that dog excrements could serve as an important breeding substrate for *L. longipalpis* in endemic areas of visceral leishmaniasis in the New World.

## Keywords

Leishmaniasis, Sand Flies, Breeding Substrate

## 1. Introduction

The leishmaniasis are diseases caused by several species of parasitic protozoan of the genus *Leishmania.* They affect around 350 million people in 88 countries and can cause basically, two types of clinical manifestations, either cutaneous or visceral.

In the cutaneous diseases, the patients show skin lesions that eventually progress to healing or evolve to serious symptoms, showing either a diffuse form or severe mucocutaneous injuries, depending on the parasite species involved.

In the visceral leishmaniasis, the parasites infect chiefly the bone marrow, liver and spleen and as a result, the patients develop a hepatosplenomegaly that may lead to death.

The main vectors of leishmanias are blood-sucking sandflies (Diptera: Psychodidae) belonging respectively two genera, *Phlebotomus* in the Old World, and *Lutzomyia* in the New World.

In Brazil and Latin America, the *Leishmania infantum* is the causative agent of visceral leishmaniasis and its most important insect vector species is the *Lutzomyia longipalpis.*

*L. longipalpis* presents a wide geographical distribution, found from southern Mexico (20˚N) to Argentina (27˚S). It has been identified in diverse ecological conditions including anthropic habitats, showing a high capability of dispersion and adaptation.

Conversely, dispersion studies also have shown that the movements of *L. longipalpis* individuals can be spatially focal and collections carried out in urban areas indicated that the complete life cycle of *L. longipalpis* occurs in peridomicile habitats [1].

Besides, studies on the flight range with this species, they agree with those above statements, showing that they exhibit a little dispersion activity in urban areas and effectively tends of remaining at the same site due to the presence of shelter and food [2].

Sandflies, differently from mosquitoes do not reproduce in water; they complete the life cycle in microhabitats exhibiting specific conditions like, organically rich moist soils, a relative high humidity and low levels of light [3].

Habitats colonized by immature forms of sandflies are typically organically rich moist soils, such as, the rain forest floor described for *Lutzomyia intermedia*, *L. umbratilis*, *L. whitmani* in the Amazon; *L. gomezi*, *L. panamensis*, *L. trapidoi* in Panama, or dirtied soil of animal shelters for *L. longipalpis* in South America, *Phlebotomus argentipes* in India; *P. chinensis* in China; *P. ariasi*, *P. perfiliewi*, *P. perniciosus* in Europe.

Emerging stages of *P. langeroni* and *P. martini* in Africa, *P. papatasi* in Eurasia and *L. longipalpis* in South America, have been observed in a wide range of ecotopes, and many species utilize rodent burrows as breeding sites, even though the importance of this niche is unclear. Larvae of some species have been found out in seemingly specific niches for instance, *L. ovallesi* on buttress roots of trees in Panama; *P. celiae* in termite hills in Kenya; *P. longipes* and *P. pedifer* in caves and among rocks in East Africa [4].

Toting up the competence of sandflies to utilize organic matter for completing its life cycle, some species were already described as breeding in dry excreta of small domestic animals and the *L. longipalpis* can also be optimally bred in the laboratory utilizing a food with rabbit feces in its composition [5].

The *L. longipalpis* shows a close relationship with the canids that are the principal mammal hosts of *L. infantum*, as in the sylvatic as well as at the domestic habitats.

Dogs play a key role on the maintenance of the parasite in nature acting as source of infection and amplification hosts. Furthermore, in endemic areas the infection of dogs precedes the human cases [6].

Anyway based on the low dispersion capacity of *L. longipalpis* in urban areas, the behavior of those insects remain at the same site due to the presence of shelter and food and their competence to reproduce in mammal feces in addition to the well-known close relationship with dogs.

In the present study, we examined under experimental conditions the potential of the dog feces as a breeding substrate to *L. longipalpis*.

## 2. Material and Methods

### 2.1. The Insects

The sand flies for all the experiments were supplied from the colony of the Laboratório de Biologia Molecular de Parasitas e Vetores from Instituto Oswaldo Cruz and they had never any previous contact with dog feces.

### 2.2. Substrates

As a substrate were utilized fresh feces pellets (2.0 cm diameter) from a five years old pug breed dog. The animal was maintained in a kennel since was born and it was duly vaccinated and dewormed. The kennel is in one area free from sandflies and leishmaniasis.

For the experiments, two conditions were observed:

1) Respectively 20 larvae from each stage from the first to fourth were put all together in plastic pots measuring 12 cm in diameter 8 cm in height. The insects were disposed around the substrate pellets taking care of avoiding artificial interactions.

The experiments proceeded with the immature forms lasted 40 days, from the onset after they were placed on the pot until end, when all of them became adult.

2) Adults, 20 females and 20 males were placed inside of the same type of pots were a pellet substrate had been previously positioned.

All the experiments were carried out in triplicate at room temperature and performed inside of a styrofoam box 20 × 28 × 18 cm, properly lined with black cardboard to keep a darkness inside.

A 40 megapixel Microsoft camera equipped with Carl Zeiss lenses was used to record the experiments make use of the time-lapse photography technics.

The same equipment was also used for filming the adult insect behavior.

The Fs showed, were some frames selected from the sequence of pictures from the original movie.

All the experiments were performed at darkness.

## 3. Results

### 3.1. Assays with the Immature Forms

Ten minutes after the beginning the tests, some larvae have already been seen near the substrate, increasing to several following 2 hours and 30 minutes (**Figure 1**).

Four hours later than tests started, several larvae were already seen around and onto the pellet with some partially buried in it. It was also possible to observe some holes made by the insects (**Figure 2**).

**Figure 1.** A top view from the pot showing in the center a dog feces pellet with some larvae surrounding it closer, at 2 hours and 30 minutes after the first contact.

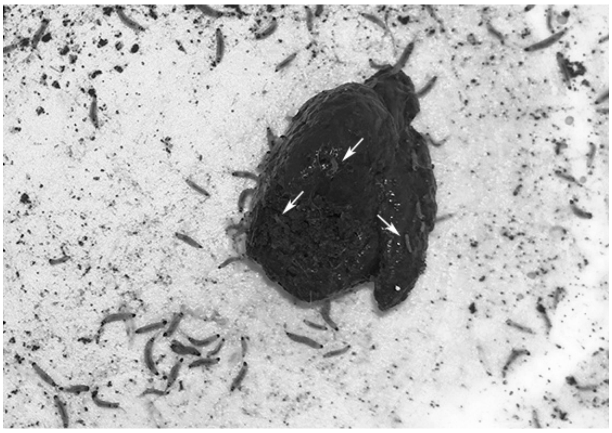

**Figure 2.** A detail of the same material at about four hours after, showing a little bit more larvae nearby the pellet, with some that have scaled it and can be seen already partially buried (arrows), magnification ×6.

Around nine hours, the same behavior was observed with an increasing of the number of insects, as around as well as onto the substrate (**Figure 3**).

At twenty-four hours, it can be seen a great quantity of immature forms covering almost completely the pellet and several of them were partially buried in the substrate forming galleries. At this time, it is also possible to see a growing white fungus on the pellet (**Figure 4**).

After just about two days (42 hours), the white fungus reached the maximum of growing, with an uncountable number of larvae completely occupying the substrate at several levels and most of them were eating the fungus (**Figure 5**).

Almost five days (117 hours) after the first contact with the substrate, the fungus was totally eaten, some galleries in the outer limits began to collapse and the few pupae have emerged (**Figure 6**).

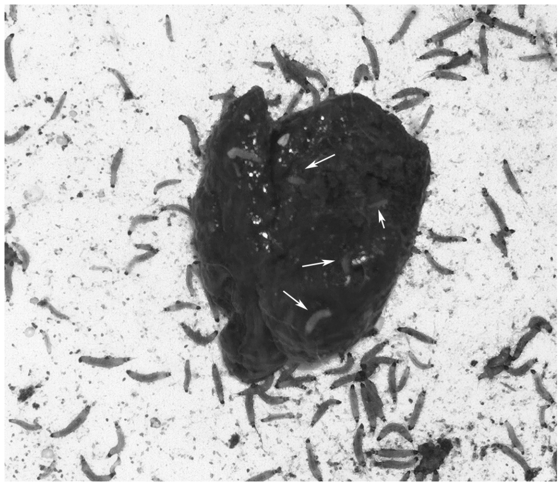

**Figure 3.** At nine hours, there was a great quantity of larvae surrounding the dog feces, with several already colonizing different sites of the top and some tunneling (arrows), magnification ×6.

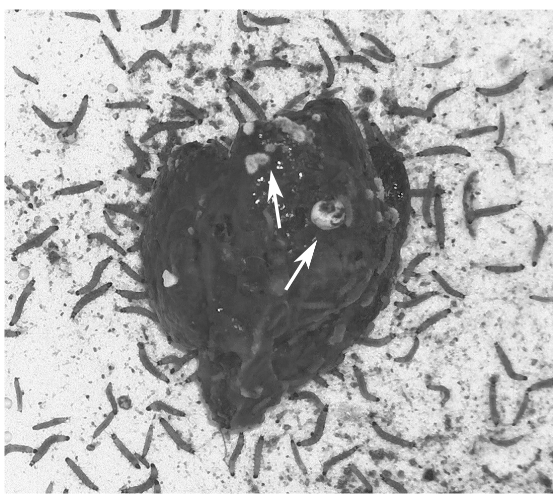

**Figure 4.** At twenty four hours, the substrate was totally colonized by larvae, but there are still numerous surrounding it. At that time, it is also possible to observe a white fungus growing (arrows), magnification ×8.

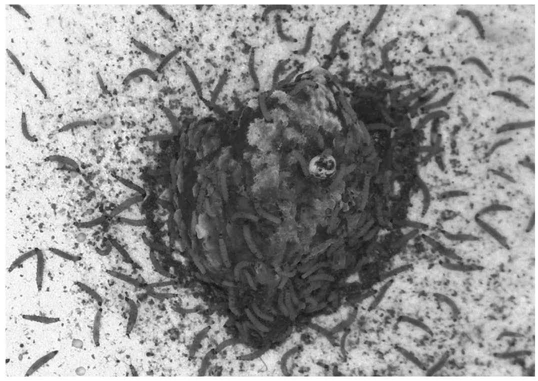

**Figure 5.** Around two days, the white fungus reached the maximum of growing and several larvae were eating it, magnification ×8.

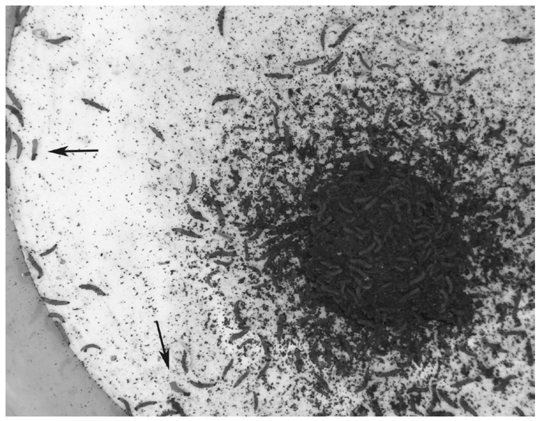

**Figure 6.** After five days, the first pupae appeared, at certain distance of the substrate (arrows), magnification ×8.

In the succeeding days, the number of pupae increased widely and most of them left the substrate and move out to the peripheral areas. The substrate was almost totally disintegrated leaving few fragments of the initial material where several remaining larvae still were feeding on it. Vestiges of the last collapsed galleries were still observed.

Along with this material, there were residues and the excrements of the larvae, as can be seen around fifteen days (361 hour) (**Figure 7**).

Finally, in the course of forty days the life cycle was completed, producing a great number of adult insects.

### 3.2. Assays with the Adult Insects

The experiments with adults lasted for fifty days to complete the whole cycle, here the immature forms originated from the eggs laid by the females showed the same behavior as observed in the previous tests initiated with larvae.

Otherwise among the adults it was observed a not yet recorded behavior until now, when males subsequently the courtship, they landed on the substrate and remained on the same place beating the wings intermittently until the death (**Figure 8**).

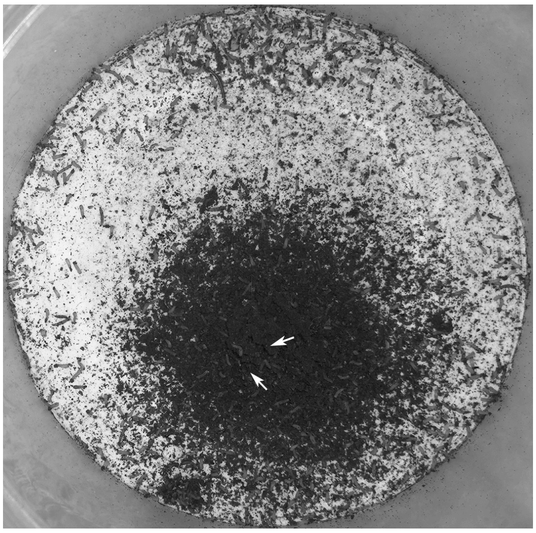

**Figure 7.** Close to fifteen days, almost all the larvae have already completed the pupation with only some eating the remaining substrate. Note some vestiges of the last collapsed galleries (arrows), magnification ×8.

**Figure 8.** Some males that had landed the substrate and were beating the wings.

## 4. Discussion

More than a century has been passed since leishmaniasis was discovered and there is still a lack of effective information about the preferred breeding sites of sand flies immature forms.

Thus, all the information concerning this subject can be very useful for the developing of further control strategies.

Our results demonstrated for the first time that *L. longipalpis* could be easily bred in dog feces, suggesting that it could represent a potential raising substrate to immature forms for this species in endemic areas of visceral leishmaniasis. Principally in peri-urban and urban environments, where the dogs are abundant and the transmission of *Leishmania infantum* exhibits a focal pattern [1] [7].

Actually, it is probable that even in the sylvatic habitats the canid feces could also have some importance on the life cycle of sand flies, since most canids select specific places to lay their feces for territory scent marking [8] and it was already observed some of those places also serving as breeding sites of sand flies [4].

We also observed a behavior that had not previously been described in plebotomines, which consisted of the larvae making galleries in the substrate, literally burying themselves in this to function also as a shelter.

Animal feces are the microhabitat for an abundant and diversified arthropod fauna [9] [10] [11] [12].

Among the coprophagous community, the dipteran are the most important decaying agents of the dung pats [10] [12] [13] [14]. Dung breeding Diptera are generally the most diverse group in cattle dung pats [11] [12] [14] [15], and several genera, including: *Musca*, *Haematobia*, *Tabanus*, *Chrysops*, *Stomoxys* among others are of veterinary importance [16] [17].

Dog excrement was already suggested as a highly productive fly-breeding medium, which may be present in considerable quantity in residential areas. It is very attractive to many species of flies and usually becomes heavily infested with fly larvae [18].

So, concerning to the *L. longipalpis* it is quite probable that they could breed in those substrates, because most areas where the visceral leishmaniasis is endemic, they correspond to poor communities sometimes with a great number of free-ranging dogs and there is no precaution about the removal of their waste.

In fact, specifically for the *L. longipalpis* under certain conditions, dog feces could be a more attractive breeding substrate, because in hookworm (*Ancylostoma caninum*), a very common nematode infecting dogs, which lay the eggs in the mammal host feces. One of the major compounds prevailing in viable and non-viable ova is the dodecanoic acid [19].

Coincidentally, the dodecanoic acid is the same component of the oviposition pheromone of *L. longipalpis* that is produced by the accessory glands and secreted onto the eggs. This pheromone attracts and/or stimulates gravid females for egg-laying [20].

One more subject related to the experiments with the larvae, included their behavior with the white fungus that have grown on the substrate. They eaten the whole fungus about 13 hours after it started to grow and there was no apparent harmful effects.

The importance of microorganisms on the diet of sand flies was already studied [21], suggested that fungi can represent an important alimentary complement for *Lutzomyia intermedia*, after observing that larva of this species usually feed on fungi. They also proposed it is possible that, as detritivores they can obtain nutrients from fungi.

Peterkova-Koci1 *et al.* (2012) recorded in *L. longipalpis* that although bacteria are not essential for a successful development, they might affect the oviposition and larval development [22]. Furthermore, different bacteria may influence the development of the insects at various degrees. *Rhizobium radiobacter* for example, can even survive the pupation and colonize the digestive tract of newly emerged females.

Telleria *et al.* (2013) suggested that *L. longipalpis* modulates defensin expression upon bacterial and *Leishmania* infection, with patterns of expression that are distinct among bacterial species and routes of infection [23].

Another significant aspect was the dispersion of most fourth instar larvae radially from the substrate before pupation. Such behavior could take place also in nature and in this case, the sites where the adults emerge would not necessarily be the same where the larval stages had developed.

In the experiments carried out with adult insects, it was observed an unprecedented behavior among the males. Under these conditions, several insects after landed on the substrate they started beating their wings intermittently, lasting until the death and remained on the same places. It is quite probably, such behavior occurred after mates, because there were already a great number of eggs on the flask walls.

A similar comportment called wing flapping was already observed before in *L. longipalpis*, associated with response to kairomones or pheromone production [24] [25].

## 5. Conclusion

Anyway, the sand flies biology principally concerning the places where the immature forms progress their development, still remains a very complex puzzle with apparently many pieces still lacking. Therefore, the confirmation of the possibility of dog feces serve as a breeding substrate to *L. longipalpis* in the endemic areas of visceral leishmaniasis, it could represent one more piece to solve it.

## Conflicts of Interest

Authors declare they have no conflicts of interest.

## Author's Contributions

JCAC—conceived the study, designed the study protocol, analysis and interpretation of these data, drafted the manuscript, and critically revised the manuscript for intellectual content.

RPB—critically revised the manuscript for intellectual content.

BSC—implementation of the data.

AVMS—designed the study protocol, analysis and interpretation of these data.

## References

[1] Casanova, C., Andrighetti, M.T.M., Sampaio, S.M.P., Marcoris, M.L.G., Colla-Jacques, F.E. and Prado, E.P. (2013) Larval Breeding Sites of *Lutzomyia longipalpis* (Diptera: Psychodidae) in Visceral Leishmaniasis Endemic Urban Areas in Southeastern Brazil. *PLoS Neglected Tropical Diseases*, **7**, 1-9. https://doi.org/10.1371/journal.pntd.0002443

[2] Brazil, R.P. (2013) The Dispersion of *Lutzomyia longipalpis* in Urban Areas. *Revista da Sociedade Brasileira de Medicina Tropical*, **46**, 263-264.

https://doi.org/10.1590/0037-8682-0101-2013

[3] Sangiorgi, B., Miranda, D.N., Oliveira, D.F., Santos, E.P., Gomes, F.R., Santos, E.O., Barral, A. and Miranda, J.C. (2012) Natural Breeding Places for Phlebotomine Sand Flies (Diptera: Psychodidae) in a Semiarid Region of Bahia State, Brazil. *Journal of Tropical Medicine*, **2012**, 1-5. https://doi.org/10.1155/2012/124068

[4] Feliciangeli, M.D. (2004) Natural Breeding Places of Phlebotomine Sandflies. *Medical and Veterinary Entomology*, **18**, 71-80. https://doi.org/10.1111/j.0269-283X.2004.0487.x

[5] Killick-Kendrick, R., Leaney, A.J. and Ready, P.D. (1977) The Establishment Maintenance and Productivity of a Laboratory Colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Journal of Medical Entomology*, **13**, 429-440. https://doi.org/10.1093/jmedent/13.4-5.429

[6] Sherlock, I.A. (1996) Ecological Interactions of Visceral Leishmaniasis in the State of Bahia, Brazil. *Memórias do Instituto Oswaldo Cruz*, **91**, 671-683. https://doi.org/10.1590/S0074-02761996000600003

[7] Silva, A.V.M., Magalhães, M.A.F.M., Brazil, R.P. and Carreira, J.C.A. (2011) Ecological Study and Risk Mapping of Leishmaniasis in an Endemic Area of Brazil Based on a Geographical Information Systems Approach. *Geospatial Health*, **6**, 33-40. https://doi.org/10.4081/gh.2011.155

[8] Barja, I. and List, R. (2014) The Role of Spatial Distribution of Faeces in Coyote Scent Marking Behavior. *Polish Journal of Ecology*, **62**, 373-384. https://doi.org/10.3161/104.062.0215

[9] Merrit, R.W. and Anderson, J.R. (1977) The Effects of Different Pasture and Rangeland Ecosystems on the Annual Dynamics of Insects in Cattle Droppings. *Hilgardia*, **45**, 31-71. https://doi.org/10.3733/hilg.v45n02p031

[10] Anderson, J.R., Merrit, R.W. and Loomis, E.C. (1984) The Insect-Free Cattle Dung Fouling of Rangeland Pastures. *Journal of Economic Entomology*, **77**, 133-141. https://doi.org/10.1093/jee/77.1.133

[11] Blume, R.R. (1985) A Checklist, Distributional Record, and Annotated Bibliography of the Insects Associated with Bovine Droppings on Pastures in America North of Mexico. *Southwest Entomology*, **9**, 1-55.

[12] Cervenka, V.J. and Moon, R.D. (1991) Arthropods Associated with Cattle Dung Pats in Minnesota. *Journal of the Kansas Entomological Society*, **64**, 131-145.

[13] Laurence, B.R. (1955) The Larval Inhabitants of Cow Pats. *Journal of Animal Ecology*, **23**, 234-260. https://doi.org/10.2307/1982

[14] Nibaruta, G. (1982) Étude écologique des diptéres associés aux excréments de la vache domestique em milieu tempéré. *Revue d'écologie et de biologie du sol*, **19**, 203-223.

[15] Poorbaugh, J.H., Anderson, J.R. and Burguer, J.F. (1968) The Insects Inhabitants of Undisturbed Cattle Droppings in Northern California. *California Vector Views*, **15**, 17-36.

[16] Lancaster, J.L. and Meisch, M.V. (1986) *Haematobia irritans* (Linneaus), the Horn Fly. In: Lancaster, J.L. and Meisch, M.V., Eds., *Arthropods in Livestock and Poultry Production*, John Wiley, New York, 94-103.

[17] Mendes, J. and Linhares, A.X. (2002) Cattle Dung Breeding Diptera in Pastures in Southeastern Brazil: Diversity, Abundance and Seasonallity. *The Memórias do Instituto Oswaldo Cruz*, **97**, 37-41. https://doi.org/10.1590/S0074-02762002000100004

[18] Wilton, D.P. (1963) Dog Excrement as a Factor in Community Fly Problems. *Proceedings of the Hawaiian Entomological Society*, **18**, 311-317.

[19] Gyawali, P., Beale, D.J., Ahmed, W., Karpe, A.V., Magalhães, R.J., Morrison, P.D. and Palombo, E.A. (2016) Determination of *Ancylostoma caninum* Ova Viability Using Metabolic Profiling. *Parasitology Research*, **115**, 3485-3492. https://doi.org/10.1007/s00436-016-5112-4

[20] Dougherty, M. and Hamilton, G. (1997) Dodecanoic Acid Is the Oviposition Pheromone of *Lutzomyia longipalpis*. *Journal of Chemical Ecology*, **23**, 2657-2671. https://doi.org/10.1023/A:1022598523803

[21] Wermelinger, E.D. and Zanuncio, J.C. (2001) Development of *Lutzomyia intermedia* and *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) Larvae in Different Diets. *Brazilian Journal of Biology*, **61**, 405-408. https://doi.org/10.1590/S1519-69842001000300009

[22] Peterkova-Koci, K., Robles-Murguia, M., Ramalho-Ortigao, M., *et al.* (2012) Leishmania Parasites with a Goal to Develop Paratransgenic Approaches for Leishmania Control. *Parasites & Vectors*, **5**, 145-153. https://doi.org/10.1186/1756-3305-5-145

[23] Telleria, E.L., Sant'Anna, M.R.V., Alkurbi, M.O., Pitaluga, A.N., Dillon, R.J. and Traub-Cseko, Y.M. (2013) Bacterial Feeding, Leishmania Infection and Distinct Infection Routes Induce Differential Defensin Expression in Lutzomyia Longipalpis. *Parasites & Vectors*, **6**, 12-20. https://doi.org/10.1186/1756-3305-6-12

[24] Bray, D.P. and Hamilton, J.G. (2007) Courtship Behaviour in the Sandfly *Lutzomyia longipalpis*, the New World Vector of Visceral Leishmaniasis. *Medical and Veterinary Entomology*, **21**, 332-338. https://doi.org/10.1111/j.1365-2915.2007.00700.x

[25] Spiegell, C.N., Dias, D.B.S., Araki, A.S., Hamilton, J.G.C., Brazil, R.P. and Jones, T.M. (2016) The Lutzomyia Longipalpis Complex: A Brief Natural History of Aggregation-Sex Pheromone Communication. *Parasites & Vectors*, **9**, 580-595. https://doi.org/10.1186/s13071-016-1866-x