

Biology of the First Generation of a Laboratory Colony of *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae)

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The phlebotomine sand flies Nyssomyia intermedia (Lutz & Neiva, 1912) and Nyssomyia neivai (Pinto, 1926) are very close and may be involved in the transmission of Leishmania spp. Ross, 1903 in Brazil. The biology of the first laboratory-reared generations of these species, descended from insects captured in Além Paraíba (N. intermedia) and Corinto (N. neivai) in the Brazilian state of Minas Gerais, is described here. The captured females were fed on hamsters and maintained individually in rearing pots. Laboratory temperature and relative humidity were maintained at 25-26°C and 80% respectively. The productivity of the first generation of N. intermedia was greater than that of N. neivai, and its development time clearly shorter, particularly for the second and third larval instars.

Key words: *Nyssomyia intermedia* - *Nyssomyia neivai* - Phlebotominae - Psychodidae

The phlebotomine sand flies *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) are very closely related species considered to be conspecific until about 10 years ago (Martins et al. 1978, Young & Duncan 1994). Males from both species can be distinguished by means of the genital filament (Andrade Filho et al. 2003) while females may be recognized by spermathecal characters such as length, number of annulae, shape and width of spermatheca head, and length of common duct in relation to individual duct (Marcondes 1996, Andrade Filho et al. 2003).

Both *N. intermedia* and *N. neivai* may be involved in the transmission of the *Leishmania* spp. Ross, 1903 causing American cutaneous leishmaniasis (ACL), particularly in Southeastern (SE) Brazil. Silva and Gomes (2001) and Gontijo et al. (2002) dissected several females of *N. intermedia* sensu lato and *N. intermedia* sensu stricto, respectively from different ACL foci but did not detect *Leishmania*. Both species bite humans avidly (Oliveira Neto et al. 1988, Gomes et al. 1989) and although *N. intermedia* s.l. has been found naturally infected with flagellates in SE Brazil (Forattini et al. 1972, Rangel et al. 1984, Casanova et al. 1995) and predominates among man-biting sand flies in several endemic or epidemic foci of ACL (Rangel et al. 1990, Gomes et al. 1995, Salomón et al. 2001, Gontijo et al. 2002), it is still only regarded as a suspected vector (Killick-Kendrick 1990), based on the fact that conspecific parasites have not been identified from isolates obtained from humans and dogs. Laboratory colonization of phlebotomine species is very important for

studies on their biology, particularly their interactions with *Leishmania* and several other pathogens (Coelho 1962, Coelho et al. 1967, Killick-Kendrick et al. 1977, Rangel et al. 1992). Species that have been successfully reared in the laboratory include *N. intermedia* s.l. (Rangel et al. 1985, 1986, Brazil et al. 1997, Ferro et al. 1998, Liutgards-Moura et al. 2000, Alves et al. 2000).

In this paper we compare the biology in the laboratory of the first generation of *N. intermedia* and *N. neivai*.

MATERIALS AND METHODS

The method for rearing of the two species were based on Killick-Kendrick et al. (1977) and Modi and Tesh (1983). The classification scheme used is that of Galati (2003).

Specimens of *N. intermedia* were collected on 27-28 August 2002 in an area known as Benjamin Constant, Além Paraíba, state of Minas Gerais (21°53'S; 42°42'W) from where Lutz and Neiva (1912) described this species. This area still retains some of the original Atlantic Forest, although cattle are grazed in clearings. Several investigators have collected sand flies in this area (Rangel et al. 1985, Marcondes 1997, Falcão pers. inform.) where *N. intermedia* is abundant. The light traps (Falcão 1981) used for sampling were hung in a cattle pen, a pigsty, a chicken house in a mango tree (used as a roosting-place by the birds) and a kennel used by a dog at night. These traps were left in place from 18:00-07:00 h. A white illuminated Shannon trap (Shannon 1939) was also hung in a peridomestic habitat of the Barra do Ouro Fino farm where sand flies were collected, by direct aspiration, from 18:00-22:00 h.

Specimens of *N. neivai* were collected in Corinto, also in the state of Minas Gerais. This collection site (17°58'S; 49°32'W) is approximately 8 km from the town of Lassance. There are several buildings in the area that are used by local residents and fishermen, fishing and agriculture being as the main economic activities of the region. The predominant vegetation type is cerrado. Sand flies were sampled using an illuminated Shannon trap in a chicken

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house localized in Lavado region, about 3 m from Rio das Velhas on July 4, 2002 from 18:00-23:00 h, this being the period of peak activity of *N. neivai* (data provided by Centro de Referência Nacional e Internacional para Flebotomíneos do Centro de Pesquisas René Rachou-Fiocruz)

After recovering the traps, sand flies were transferred to 11 cm diameter rearing containers, each lined with plaster to maintain humidity and with a hole in the base plugged with plaster, that could be placed on a damp substrate. The containers were topped with nylon mesh with a hole cut in the centre, through which sand flies were introduced using a Castro aspirator (Castro pers. inform. by Barretto & Coutinho 1940 apud Pessoa & Barretto 1948). When not in use the hole was plugged with cotton wool. A cotton wool swab soaked in sugar solution was placed on the mesh as an energy source.

Sand flies were fed in the field on hamsters [*Mesocricetus auratus* (Water House, 1839)] anaesthetised with 0.7-0.8 ml Thiopental (1 g/20 ml distilled water) and placed directly on top of the containers, allowing sand flies to feed through the mesh. Rearing containers containing the sand flies were transported from the collection site to the laboratory in Tupperware® boxes lined with moistened paper towels.

In the laboratory, the sand flies were allowed to feed on hamsters a second time. Females that survived 48 h after blood feeding were then transferred to individual (oviposition) vials 4 cm in diameter.

Cotton wool swabs soaked in fresh sugar solution were placed on the mesh of the oviposition vials. The vials were inspected daily to check whether the females had laid eggs. Females that had oviposited and were still alive were given the opportunity to take a new blood meal from a hamster, Japanese quail (*Coturnix coturnix japonica* Temminck and Schlegel, 1849) or human volunteer.

After each female sand fly died, it was mounted in Berlese fluid on a microscope slide labelled with the same number as the oviposition vial. These preparations were examined under the microscope for species identification.

Progeny of the wild-caught insects were reared in the laboratory at 25-26°C and relative humidity of approximately 80%. The oviposition vials containing eggs were monitored daily and after larvae emerged they were fed with a powdered diet containing equal proportions of "xaxim" (an arboreal Pteridophyta), dry rabbit faeces, dehydrated lettuce, and Vitormonio® fish food. The vials were examined daily to monitor possible contamination by fungi or mites and to remove excess faeces or leftover food.

The numbers of eggs per female, larvae in each stage, pupae, and adults were noted as development progressed. Developmental parameters of *N. intermedia* and *N. neivai* such as incubation periods, duration of larval stages and adult eclosion were calculated as averages of times from the first to the final day of the stage in question.

RESULTS

Data obtained for *N. intermedia* and *N. neivai* concerning sex ratios, productivity and development times are compared in Figs 1 and 2.

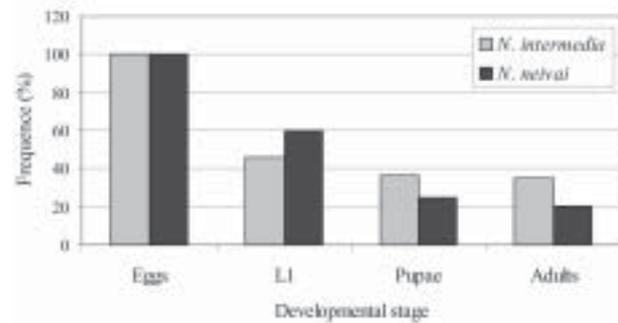


Fig. 1: percentage oviposition, hatching rate of first instar larvae (L1), pupae, and adults emerged of the first laboratory generations of *Nyssomyia intermedia* and *N. neivai* from Minas Gerais, Brazil

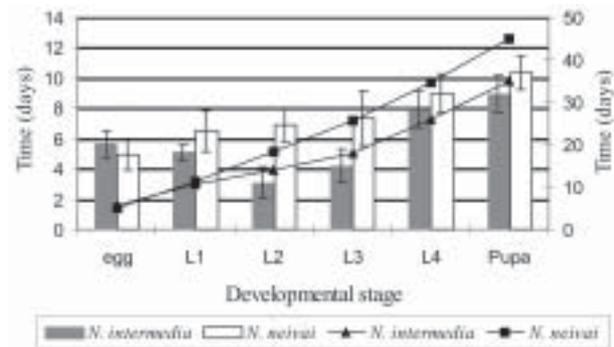


Fig. 2: development periods of the first laboratory generations of *Nyssomyia intermedia* and *N. neivai* from Minas Gerais, Brazil

Nyssomyia intermedia - Field-collected females fed actively on hamsters and also tried to bite man although they were not allowed to do so. A total of 123 females collected in Além Paraíba were separated into individual oviposition/rearing chambers and 72 of these (58.5%) laid eggs. A single female that did not belong to either of the two species being studied was identified as *Nyssomyia whitmani* (Antunes & Coutinho, 1939). The percentage of *N. intermedia* females that laid eggs was 57.7% and eggs hatched in all of the rearing chambers. Thirty-nine (54.2%) of the females that laid eggs survived oviposition but none of them took a second blood meal. The total number of eggs laid was 3228, a mean of 45.5 per female (range 12-78). From these 1474 first instar (L1) larvae were obtained, representing a hatch rate of 45.7%. A total of 1144 adults emerged (35.4% of the eggs and 77.6% of larvae hatched) in 60/72 (82.3%) of the rearing chambers where oviposition occurred of these 51.5% were females and 48.5% males (M:F sex ratio 0.94:1).

Male sand flies were the first to emerge. The entire egg to adult period ranged from 29 to 42 days (average 34.9 days) for *N. intermedia*. Average duration of the egg stage was 5.7 days, while duration of each larval stage (in days) was as follows: L1 5.1; L2 3.1; L3 4.2, and L4 7.9. The pupal stage was the most prolonged, lasting a mean of 9.0 days.

Nyssomyia neivai - As with *N. intermedia*, *N. neivai* females fed readily on hamsters. They also bit man, but not japonica quail, when these were offered as feeding sources. A total of 91 females collected in Corinto were separated into individual oviposition/rearing chambers. One of the 54 *N. neivai* rearing chambers was found to contain a different species, identified as *Evandromyia lenti* (Mangabeira, 1938). Only eight (15.1%) of the remaining 53 females survived oviposition and these did not take another blood meal. A total of 2573 eggs was laid by the 53 *N. neivai* females, a mean of 48.5 per female, with up to 70 eggs in a single batch. The 2573 eggs produced 1552 L1 larvae, a hatch rate of 60.3%. Adult sand flies were produced from 39/53 (73.6%) of the oviposition/rearing chambers. Adult survival rate as a proportion of eggs laid was 533/2573 (20.7%) while 533/1552 (34.3%) of L1 larvae completed development. The M:F sex ratio was 43.3/56.7% (0.76:1)

Males were the first to emerge, as in *N. intermedia*. The entire egg to adult period for *N. neivai* was 45.1 days (range 36-54). Average duration for the egg stage was 5 days. There was little variation between any of the first three larval stages: the L1 lasted a mean of 6.5 days, L2 6.9, and L3 7.3. The L4 and pupal stages lasted a mean of 9.0, and 10.4 days respectively.

DISCUSSION

The fact that 99% of all sand flies collected in Benjamin Constant and Corinto were either *N. intermedia* or *N. neivai* indicates that these areas may be used to provide more material for laboratory rearing studies in the future.

Under laboratory conditions, *N. intermedia* females remained alive after oviposition, unlike those of *N. neivai*, which had a low survival rate. This suggests that the former species would be more important in *Leishmania* transmission, since it would be able to take a second blood meal.

The fact that none of the insects re-fed in the laboratory, even when offered the chance to bite hamsters, japonica quail or humans, may have been due to the fact that they were held individually, and thus were isolated from males or other females whose presence might stimulate blood feeding.

Gomes et al. (1982) reported that *N. intermedia* s.l. fed twice under natural conditions. This was also observed by Brazil et al. (1991) for *N. intermedia* and Dia-Eldin et al. (1992) for *Lutzomyia longipalpis* (Lutz and Neiva, 1912), both in nature and in the laboratory. In the wild, *N. intermedia* has also been reported to survive after oviposition (Gomes et al. 1982). Sand fly survival is rare under laboratory conditions, the few records involving very low rates, including approximately 17.7% for *Lutzomyia renei* (Martins Falcão & Silva, 1957) (Alves et al. 2001) and 8.3% for *L. longipalpis* (Souza et al. 1995). According to Casanova (2001), *N. neivai* would be able to feed up to three times within 10 days.

The percentage of individualized females that oviposited was nearly the same for both species under study, at 57.5% for *N. intermedia* and 59.3% for *N. neivai*. Such values correspond closely with those reported by Rangel

et al. (1985), who worked with four generations of *N. intermedia*, in which 58.9% of blood-fed females laid eggs.

Casanova (2001) found that *N. neivai* fed mostly on human blood in peridomestic areas, although he considered this species to be opportunistic rather than truly anthropophilic, feeding preference varying with host availability. Aguiar et al. (1987) arrived at similar conclusions for *N. intermedia* in Itaguaí in the Brazilian state of Rio de Janeiro. According to Rangel et al. (1986), the number of *N. intermedia* females that laid eggs and the number of eggs per female depend on the blood source.

The mean number of eggs laid per female was quite similar for both species, although slightly higher for *N. neivai*. The means found here are higher than those observed by Rangel et al. (1985), who recorded 40.2 eggs per *N. intermedia* female in the first generation, or by Alves et al. (2001) who reported 15.7 eggs per female in field-collected *L. renei*. The mean was also significantly higher than the 32.7 eggs per female observed for *Evandromyia carmelinoi* (Ryan, Fraiha, Lainson, Shaw, 1986) (Alves et al. 2000).

During the present study the hatch rate in *N. neivai* was 60.3%, much higher than that of *N. intermedia* (45.7%). Similar values were found for *N. intermedia* by Rangel et al. (1985), i.e., 64.1%. Values for both species are much higher than those of *L. renei*, which showed a hatching rate of 24% (Alves et al. 2001).

The productivity of *N. intermedia* was higher than that of *N. neivai*, the percentage of eggs that ultimately gave rise to adults in the former being 35.4%. In all, 77.6% of the L1 larvae reached the adult stage, representing a very low mortality rate during development. The corresponding values for *N. neivai* were much lower, at 20.7% and 34.3% respectively. It is noteworthy that the highest mortality rate among phlebotomine sand flies occurs between the first and second developing stages and that these developmental stages were more prolonged in *N. neivai* than in *N. intermedia*, particularly L2. This might have affected the mortality rate at this stage. In *L. renei* only 8.6% of the eggs and 35.6% of the L1 larvae ultimately gave rise to adults (Alves et al. 2001). Rangel et al. (1986) recorded much higher indices for *N. intermedia* at 34.4% and 53.6% respectively.

The M:F ratio was higher for *N. intermedia*, although Rangel et al. (1985) found that 45.1% of the emerging adults of this species in the laboratory were females. Males emerged before females, as has been reported by other authors (Chaniotis & Anderson 1964, Killick-Kendrick et al. 1977, Montoya-Lerma et al. 1998, Alves et al. 2000).

N. neivai had a longer life cycle than *N. intermedia*, the two species requiring 45 and 35 days respectively to complete development. This fact might be due to the longer developing period of second and third instar larvae, an average of 7 days for each stage, whereas for *N. intermedia* the media was 7 days to complete both phases (3 days for L2 and 4 for L3). Developing periods for immature stages of both species have been shown to be higher than for *L. longipalpis*, lasting 29.5 days (Brazil et al. 1997). According to Rangel et al. (1985) the entire cycle for the first generation of *N. intermedia* lasted 37 days, very close to the results of the present study and a little longer than the

32.8 days reported by Brazil et al. (1997). The development period of *N. neivai* was close to that of *E. lenti*, which requires 40.2 days (Brazil et al. 1997). The longest developmental stages were the L4 and pupa, both of which involve more radical physiological changes and tissue formation than previous instars (Forattini 1973).

Several physical factors may affect phlebotomine biological cycles (Rangel et al. 1987, Nieves et al. 1997), besides biological factors such as larval diet (Rangel et al. 1985, Wermelinger & Zanúncio 2001). Attempts during the present study to maintain colonies of *N. intermedia* and *N. neivai* in larger rearing chambers were unsuccessful, since F2 females died just after taking blood meals. It may be that symbiotic protozoa or bacteria, possibly acquired from natural diet during larval instars or sugar meals of females, assist in bloodmeal digestion. These are not present in the larval diet or sugar meals provided to adult females in the laboratory and this may explain the low survival rate of the insects. Studies should be performed on intestinal microbiota of wild-caught *N. intermedia* and *N. neivai* in order to provide further information on this aspect of their biology, as has already been done for *L. longipalpis* (Oliveira et al. 1999, 2001).

REFERENCES

- Aguiar GM, Vilela ML, Lima RB 1987. Ecology of the sandflies of Itaguaí, an area of cutaneous leishmaniasis in the state of Rio de Janeiro. Food preferences (Diptera, Psychodidae, Phlebotominae). *Mem Inst Oswaldo Cruz* 82: 583-584.
- Alves JCM, Pacheco RFS, Brazil RP 2001. Aspectos biológicos de *Lutzomyia renei* (Martins, Falcão & Silva, 1957) (Diptera, Psychodidae) em campo e em laboratório. *Rev Bras Entomol* 45: 17-21.
- Alves JCM, Pedras, MJ, Andrade Filho JD, Brazil BG, Souza AA, Brazil RP 2000. Biology of *Lutzomyia carmelinoi* Ryan, Fraiha, Lainson & Shaw (Diptera: Psychodidae) under laboratory conditions. *Entomol Vect* 7: 281-286.
- Andrade Filho JD, Galati EAB, Falcão AL 2003. Redescription of *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae). *Mem Inst Oswaldo Cruz* 98: 1059-1065.
- Brazil RP, Almeida DC, Brazil BG, Oliveira Mamede SM 1991. Chicken house as a resting site of sandflies in Rio de Janeiro, Brazil. *Parassitology* 33 (Suppl.): 113-114.
- Brazil RP, Carneiro VL, Andrade Filho JD, Alves JCM, Falcão AL 1997. Biology of *Lutzomyia lenti* (Mangabeira) (Diptera: Psychodidae). *An Soc Entomol Bras* 26: 191-193.
- Casanova C 2001. *Avaliação do Potencial Bbiológico de Lutzomyia intermedia s.l. como Vetor da Leishmaniose Tegumentar Americana na Zona Rural do Município de Conchal, Estado de São Paulo*, Thesis, Universidade de São Paulo, São Paulo, 99 pp.
- Casanova C, Mayo RC, Rangel O, Mascarini LM, Pignatti MG, Galati EAB, Gomes AC 1995. Natural *Lutzomyia intermedia* (Lutz & Neiva) infection in the valley of the Mogi Guaçu River, State of São Paulo, Brazil. *Bol Dir Malariol San Amb* 35 (Suppl. 1): 77-84.
- Chaniotis BN, Anderson JR 1964. Notes on the morphology and laboratory rearing of *P. v. occidentis*. *Pan-Pacif Entomol* 40: 27-32.
- Coelho MV 1962. Suscetibilidade de *Phlebotomus longipalpis* e *P. renei* à infecção por *Leishmania braziliensis*. *Rev Inst Med Trop São Paulo* 4: 101-104.
- Coelho MV, Falcão AR, Falcão AL 1967. Desenvolvimento de espécies do gênero *Leishmania* em espécies brasileiras do gênero *Lutzomyia* França, 1924. V - Infectividade de leptomomas evoluindo no flebótomo e experiências de transmissão de leishmanioses. *Rev Inst Med Trop São Paulo* 9: 367-373.
- Dia-Eldin AE, Morton J, Brazil RP, Ward RD 1992. Field and laboratory evidence for multiple blood feeding by *Lutzomyia longipalpis* (Diptera: Psychodidae). *Med Vet Entomol* 6: 173-174.
- Falcão AR 1981. Um novo modelo de armadilha luminosa de sucção para pequenos insetos. *Mem Inst Oswaldo Cruz* 76: 303-305.
- Ferro C, Cardenas E, Corredor D, Morales A, Munstermann LE 1998. Life cycle and fecundity analysis of *Lutzomyia shannoni* (Dyar) (Diptera: Psychodidae). *Mem Inst Oswaldo Cruz* 93: 195-199.
- Forattini OP 1973. *Entomologia Médica*, Edgard Blucher, São Paulo, 658 pp.
- Forattini OP, Pattoli DBG, Rebello EX, Ferreira AO 1972. Infecção natural de flebotomíneos em foco enzoótico de leishmaniose no estado de São Paulo, Brasil. *Rev Saúde Pública* 6: 431-433.
- Galati EAB 2003. Classificação de Phlebotominae. In EF Rangel, R Lainson (eds), *Flebotomíneos do Brasil*, Fiocruz, Rio de Janeiro, p. 23-51.
- Gomes AC, Barata JMS, Rocha e Silva EO, Galati EAB 1989. Aspectos ecológicos da leishmaniose tegumentar americana. 6. Fauna flebotomínea antropofílica de matas residuais situadas na região centro-nordeste do estado de São Paulo. *Rev Inst Med Trop São Paulo* 31: 32-39.
- Gomes AC, Galati EAB, Casanova C, Domingos MF, Marques GRAM, Neves VLF 1995. Analysis of the geographical distribution of leishmaniasis vectors in the state of São Paulo Brazil. *Bol Dir Malariol San Amb* 35 (Supl. 1): 143-146.
- Gomes AC, Rabello EX, Santos JLF, Galati EAB 1982. Aspectos ecológicos da leishmaniose tegumentar americana. 2. Ecótopos artificiais como abrigo de *Psychodopygus intermedius* e observações sobre alimentação e reprodução sob influência de fatores físicos naturais. *Rev Saúde Pública* 16: 149-159.
- Gontijo CMF, Silva ES, Fuccio MB, Sousa MCA, Pacheco RS, Dias ES, Andrade Filho JD, Brazil RP, Melo MN 2002. Epidemiological studies of an outbreak of cutaneous leishmaniasis in the Rio Jequitinhonha Valley, Minas Gerais, Brazil. *Acta Trop* 81: 143-150.
- Killick-Kendrick R 1990. Phlebotomine vectors of the leishmaniasis: a review. *Med Vet Entomol* 4: 1-24.
- Killick-Kendrick R, Leaney AJ, Ready PD 1977. The establishment, maintenance and productivity of a laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). *J Med Entomol* 13: 429-440.
- Luitgards-Moura JF, Castellón-Bermúdez EG, Rosa-Freitas MG 2000. Aspects related to productivity for four generations of a *Lutzomyia longipalpis* laboratory colony. *Mem Inst Oswaldo Cruz* 95: 251-257.
- Lutz A, Neiva A 1912. Contribuição para o conhecimento das espécies do gênero *Phlebotomus* existentes no Brasil. *Mem Inst Oswaldo Cruz* 4: 84-95.
- Marcondes CB 1996. A redescription of *Lutzomyia* (*Nyssomyia*) *intermedia* (Lutz & Neiva, 1912), and resurrection of *L. neivai* (Pinto, 1926) (Diptera, Psychodidae, Phlebotominae). *Mem Inst Oswaldo Cruz* 91: 457-462.
- Marcondes CB 1997. *Morfometria e DNA Mitochondrial de Populações Sul Americanas de Lutzomyia* (*Nyssomyia*) *intermedia* (Lutz & Neiva, 1912) (Diptera, Psychodidae, Phlebotominae), Thesis, Universidade Federal do Paraná, Curitiba, 260 pp.
- Martins AV, Williams P, Falcão AL 1978. *American Sandflies*

- (Diptera: Psychodidae: Phlebotominae), Acad Bras Ciências, Rio de Janeiro, 195 pp.
- Modi GB, Tesh RB 1983. A simple technique for mass rearing *Lutzomyia longipalpis* and *Phlebotomus papatasi* (Diptera: Psychodidae) in the laboratory. *J Med Entomol* 20: 568-569.
- Montoya-Lerma J, Caldena-Peña H, Jaramillo-Salazar C 1998. Rearing and colonisation of *Lutzomyia evansi* (Diptera: Psychodidae), a vector of visceral leishmaniasis in Colombia. *Mem Inst Oswaldo Cruz* 93: 263-268.
- Nieves E, Ribeiro A, Brazil RP 1997. Physical factors influencing the oviposition of *Lutzomyia migonei* (Diptera: Psychodidae) in laboratory conditions. *Mem Inst Oswaldo Cruz* 92: 733-737.
- Oliveira SMP, Loureiro MM, Leandro MJF, Giordano-Dias CM, Brazil RP, Almeida JM, Asensi MD, Mello RP 1999. Caracterização da microbiota do trato digestivo em fêmeas de *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae) alimentadas com frutose. *Entomol Vect* 6: 166-179.
- Oliveira SMP, Morais BA, Gonçalves CA, Giordano-Dias CM, Vilela ML, Brazil RP, Almeida JM, Asensi MD, Mello RP 2001. Microbiota do trato digestivo de fêmeas de *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae) provenientes de colônias alimentadas com sangue e com sangue e sacarose. *Cad Saúde Pública* 17: 229-232.
- Oliveira Neto MP, Pirmey C, Rangel EF, Schubach A, Grimaldi G 1988. An outbreak american cutaneous leishmaniasis (*Leishmania braziliensis braziliensis*) in a periurban area of Rio de Janeiro City, Brazil: clinical and epidemiological studies. *Mem Inst Oswaldo Cruz* 83: 427-435.
- Pessoa SB, Barreto MP 1948. *Leishmaniose Tegumentar Americana*, Imprensa Nacional, Rio de Janeiro, 527 pp.
- Rangel EF, Azevedo ACR, Andrade CA, Souza NA, Wermelinger ED 1990. Studies on sandfly fauna (Diptera: Psychodidae) in a focus of cutaneous leishmaniasis in Mesquita, Rio de Janeiro State, Brazil. *Mem Inst Oswaldo Cruz* 85: 39-45.
- Rangel EF, Barbosa AF, Andrade CA, Souza NA, Wermelinger ED 1992. Development of *Leishmania (viannia) braziliensis* Vianna, 1911 in *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae) under experimental conditions. *Mem Inst Oswaldo Cruz* 87: 235-238.
- Rangel EF, Souza NA, Wermelinger ED, Barbosa AF 1984. Infecção natural de *Lutzomyia intermedia* Lutz & Neiva, 1912 em área endêmica de leishmaniose tegumentar no estado do Rio de Janeiro. *Mem Inst Oswaldo Cruz* 79: 395-396.
- Rangel EF, Souza NA, Wermelinger ED, Barbosa AF 1985. Estabelecimento de colônia, em laboratório, de *Lutzomyia intermedia* Lutz & Neiva, 1912 (Diptera, Psychodidae, Phlebotominae). *Mem Inst Oswaldo Cruz* 80: 219-226.
- Rangel EF, Souza NA, Wermelinger ED, Barbosa AF 1987. Biologia de *Lutzomyia intermedia* Lutz & Neiva, 1912 e *Lutzomyia longipalpis* Lutz & Neiva, 1912 (Diptera, Psychodidae), em condições experimentais. II. Influência de fatores ambientais no comportamento das formas imaturas e adultas. *Mem Inst Oswaldo Cruz* 83: 385-394.
- Rangel EF, Souza NA, Wermelinger ED, Barbosa AF, Andrade CA 1986. Biologia de *Lutzomyia intermedia* Lutz & Neiva, 1912 e *Lutzomyia longipalpis* Lutz & Neiva, 1912 (Diptera, Psychodidae), em condições experimentais. I Aspectos da alimentação de larvas e adultos. *Mem Inst Oswaldo Cruz* 81: 431-438.
- Silva AC, Gomes AC 2001. Estudo da competência vetorial de *Lutzomyia intermedia* (Lutz & Neiva, 1912) para *Leishmania (Viannia) braziliensis*, Vianna, 1911. *Rev Soc Bras Med Trop* 34: 187-191.
- Salomon OD, Zaidenberg M, Burgos R, Heredia VI, Caropresi SL 2001. American cutaneous leishmaniasis outbreak, Tartagal city, province of Salta, Argentina, 1993. *Rev Inst Med Trop São Paulo* 43: 105-108.
- Shannon RC 1939. Methods for collecting and feeding mosquitoes in jungle yellow fever studies. *Am J Trop Med Hyg* 19: 131-148.
- Souza NA, Andrade Coelho CA, Barbosa AF, Vilela ML, Rangel EF, Deane MP 1995. The influence of sugar and amino acids on the blood-feeding behaviour, oviposition and longevity of laboratory colony of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae, Phlebotominae). *Mem Inst Oswaldo Cruz* 90: 751-757.
- Wermelinger ED, Zanuncio JC 2002. Development of *Lutzomyia intermedia* and *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) larvae in different diets. *Braz J Biol* 61: 405-408.
- Young DG, Duncan MA 1994. *Guide to the Identification and Geographic Distribution of Lutzomyia Sand Flies in the Mexico, the West Indies, Central and the South America (Diptera: Psychodidae)*. Mem Am Entomol Inst 54, Associated Publishers, 881 pp.

