Phlebotomine sandfly ecology on the Indian subcontinent: does village vegetation play a role in sandfly distribution in Bihar, India?

D. M. POCHÉ¹, R. M. POCHÉ¹, S. MUKHERJEE², G. A. FRANCKOWIAK¹, L. N. BRILEY¹, D. J. SOMERS¹ and R. B. GARLAPATI²

¹Genesis Laboratories, Inc., Wellington, CO, U.S.A. and ²Genesis Laboratories, India Private Ltd, Patna, India

Abstract. Visceral leishmaniasis (VL) is a disease that results in approximately 50,000 human deaths annually. It is transmitted through the bites of phlebotomine sandflies and around two-thirds of cases occur on the Indian subcontinent. Indoor residual spraying (IRS), the efficacy of which depends upon sandfly adults resting indoors, is the only sandfly control method used in India. Recently, in Bihar, India, considerable sandfly numbers have been recorded outdoors in village vegetation, which suggests that IRS may control only a portion of the population. The purpose of this study was to revisit previously published results that suggested some sandflies to be arboreal and to rest on outlying plants by using Centers for Disease Control light traps to capture sandflies in vegetation, including banana plants and palmyra palm trees, in two previously sampled VL-endemic Bihari villages. Over 3500 sandflies were trapped in vegetation over 12 weeks. The results showed the mean number of sandflies collected per trap night were significantly higher in banana trees than in other vegetation (P = 0.0141) and in female rather than male palmyra palm trees (P = 0.0002). The results raise questions regarding sandfly dispersal, oviposition and feeding behaviours, and suggest a need to refine current control practices in India and to take into account an evolving understanding of sandfly ecology.

Key words. Borassus flabellifer, Musa acuminata, Phlebotomus argentipes, banana plants, breeding sites, CDC light traps, dispersal, palmyra palm trees, vector control, visceral leishmaniasis.

Introduction

Visceral leishmaniasis (VL) is a devastating disease that affects the poorest of the poor (Boelaert et al., 2009). It is responsible for approximately 50,000 human infections and around 50,000 human fatalities each year, and is regarded as the second most deadly parasitic killer after malaria (Desjeux, 2001a, 2001b). The Indian subcontinent represents the largest VL focus in the world (Alvar et al., 2012) and accounts for around 67% of reported VL cases (Chappuis et al., 2007), in which Leishmania donovani (Kinetoplastida: Trypanosomatidae) is the incriminated pathogen (Singh et al., 2006). In India, around 90% of VL cases occur in the impoverished state of Bihar (Singh et al., 2006). Visceral leishmaniasis is transmitted through the bites of phlebotomine sandflies, small dipterans that rarely exceed 3 mm in length (Killick-Kendrick, 1999), of which Phlebotomus argentipes (Diptera: Psychodidae) is the only incriminated VL vector on the Indian subcontinent (Dinesh et al., 2000). Vector control through the use of insecticides may prove paramount in reducing sandfly numbers and subsequently reducing VL transmission in India. However, the organization and implementation of effective vector control strategies are
and palmyra palm trees (Poché et al. selected. Phlebotomus argentipes adults have been thought to be endophilic and endophagic, resting and feeding indoors, which is why indoor residual spraying (IRS) is the only vector control practice used in Bihar (Bern et al., 2010). However, recent research suggests that at least a portion of P.argentipes may be exophilic and exophagic, resting and feeding outdoors. A study conducted over a 13-month period in three Bihar villages by Poché et al. (2011) found that over 30% of the nearly 53,000 sandflies collected were found outdoors in vegetation. Of 288 blood-fed sandflies found to be polymerase chain reaction-positive to cytochrome b amplification, the majority (∼90%) were determined to have blood-fed on either humans, cows (Bos taurus, Bos indicus) or domestic buffalo (Bubalus bubalis), and approximately 26% of blood-fed sandflies were collected in outlying vegetation (Garlapati et al., 2012).

The role of vegetation was also examined during a study in which over 5000 sandflies were collected from the canopies of palmyra palm trees (Borassus flabellifer) at up to 18.4 m above the ground (Poché et al., 2012), a result which indicated that P. argenteipes, although generally believed to be a weak flyer, is quite capable of vertical dispersal. The high number of sandflies distributed outdoors suggests that IRS may control only a portion of the sandfly population, given that its success depends on sandflies being endophilic (Coleman et al., 2015).

The purpose of this study was to follow up on prior sandfly collections performed in outlying village vegetation (Poché et al., 2011) and palmyra palm trees (Poché et al., 2012) in order to provide further evidence of exophilic sandfly behaviour in Bihar. This involved the use of Centers for Disease Control (CDC) light traps to capture sandflies in outlying village vegetation and palmyra palm trees. The hypothesis was that sandflies would be found in peri-domestic vegetation, as they were in the earlier studies (Poché et al., 2011, 2012). This research may encourage managers to pursue means of control that explicitly target outdoor resting sandflies in order to complement the practice of targeting indoor resting sandflies through IRS.

Materials and methods

Study areas

The study was conducted in the Saran District, Bihar, India, approximately 30 km northwest of Patna. Two villages, Mahesia and Sutihaar, in which sandflies had been previously collected from outlying village vegetation (Poché et al., 2011) and palmyra palm trees (Poché et al., 2012), respectively, were selected.

Mahesia. The socioeconomic status of Mahesia (25°50' N, 84°57' E) is low (<US$500 per year per household). The majority of homes are constructed of thatch and brick and members of over 60% of households own livestock comprised mainly of cattle, buffalo, goats and chickens (Poché et al., 2011). The village is highly agricultural and is surrounded by outlying vegetation including various vegetables and fruits. It is considered VL-endemic, with the three most recent cases being reported in June (n = 1) and December (n = 2) 2014.

Sutihaar. The socioeconomic status of Sutihaar (25°51' N, 84°59' E) is very low and is reflected in a high unemployment rate. The majority of homes are constructed of thatch and brick. Goats are the most abundant form of livestock. Wild rodents such as Rattus rattus and Bandicota bengalensis are also common. Outlying vegetation is highly abundant in this village, which is populated by over 500 palmyra palm trees, commonly used by villagers to produce palm wine (toddy). The village is VL-endemic and numerous cases have been reported in recent years.

Sandfly monitoring

Sandfly collection was performed between 23 September and 9 December 2015. After receiving permission from landowners, CDC light traps (Bioquip Products, Inc., Compton, CA, U.S.A.), powered by rechargeable 6-V batteries, were positioned in outlying vegetation in Mahesia and in palmyra palm trees in Sutihaar. A protective cover was fitted to each trap to shield electronic components from rain and falling debris. The location of each CDC light trap was recorded using a handheld GPS (eTrex 30®; Garmin International, Inc., Olathe, KS, U.S.A.). Traps were set at around sunset and removed at around sunrise the following morning. Trap catches were then transported to Genesis Laboratories Pvt. Ltd in Patna and stored at −20°C. Collected sandflies were separated from other insects, counted and morphologically identified according to sex and to one of the genera Phlebotomus or Sergentomyia (Diptera: Psychodidae). Only Phlebotomus sandflies were further identified to species level because this genus includes species incriminated as VL vectors.

Mahesia. In Mahesia, five CDC light traps were positioned in five outlying vegetation locations, with the light source positioned at approximately 1.0 m above the ground (Poché et al., 2011). The vegetation type at each trap was noted and found to consist largely of maize and various fruits. The diversity of vegetation at each CDC light trap location is indicated in Table 1.

Sutihaar. In Sutihaar, 12 CDC light traps were positioned in the canopies of six male and six female palmyra palm trees (one CDC light trap per tree). The locations were selected based on the availability of the owners, who gave permission to set the traps. Once permission had been granted, two hired climbers set traps in the canopies of the trees at around 0.5–1.0 m away from the trunk (Figs 1 and 2). The heights of the palm trees ranged from 10.9 to 13.7 m (mean height: 12.2 m) (Table 2). Study personnel were present for the setting and collection of CDC light traps to ensure that traps were set in the canopies of the trees.
Table 1. Vegetation types present (X) at each of the five Centers for Disease Control light trap locations (trap nos. GLE-1–GLE-5) from which sandflies were collected during September–December 2015 in the village of Mahesia.

| Vegetation type                        | GLE-1 | GLE-2 | GLE-3 | GLE-4 | GLE-5 |
|----------------------------------------|-------|-------|-------|-------|-------|
| Mango (Mangifera indica)               | X     |       | X     |       |       |
| Guava (Psidium guajava)                | X     | X     | X     | X     |       |
| Litchi (Litchi chinensis)              | X     | X     |       |       |       |
| Citrus (Citrus spp.)                   | X     | X     |       |       |       |
| Maize (Zea mays)                       | X     | X     |       | X     | X     |
| Papaya (Carica papaya)                 | X     |       |       |       |       |
| Banana (Musa acuminata)                | X     |       |       |       |       |
| Teak (Tectona grandis)                 |       |       | X     |       |       |
| Jackfruit (Artocarpus heterophyllus)   |       |       |       | X     |       |
| Sponge gourd (Luffa aegyptiaca)        |       |       | X     |       |       |
| Castor oil plant (Ricinus communis)    |       |       |       |       | X     |
| Indian fig tree (Ficus racemosa)       |       |       |       |       | X     |
| Palm tree (Borassus flabellifer)       |       |       |       |       | X     |
| Beechwood (Gmelina arborea)            |       |       |       |       | X     |
| Drumstick tree (Moringa oleifera)      |       |       |       |       | X     |
| Bottle gourd (Lagenaria siceraria)     |       |       |       |       | X     |
| Bodhi tree (Ficus religiosa)           |       |       |       |       | X     |
| Bamboo (Subfamily: Bambusoideae)       |       |       |       |       | X     |

Fig. 1. A hired climber setting a Centers for Disease Control light trap in a palmyra palm tree.

Data analysis

The mean number of sandflies per trap night in palms and vegetation was determined for each collection date. Differences in median values between the numbers of _P. argentipes_ and _Sergentomyia_ spp. per trap night collected from each village, between the numbers of sandflies per trap night collected from different vegetation types in Mahesia and, lastly, between the numbers of sandflies per trap night collected from male and female palmyra palm trees in Sutihaar, were assessed using a non-parametric Wilcoxon rank sum test (\(P \leq 0.05\)), with date of collection as a blocking variable.

Results

Sandfly captures

From 23 September 2015 to 9 December 2015 (12 weeks) a total of 3550 sandflies were collected over 204 trap nights.

A total of 1490 _P. argentipes_ (842 males, 648 females), 2058 _Sergentomyia_ spp. (735 males, 1323 females), and two _Phlebotomus papatasi_ (two males) were collected and morphologically identified.

Mahesia: outlying vegetation captures. In total, 1764 sandflies were collected from outlying vegetation over 60 trap nights (29.4 sandflies/trap night) (Fig. 3A). Numbers of _P. argentipes_ (20.98/trap night) collected in vegetation were markedly higher than those of _Sergentomyia_ spp. (8.42/trap night), which supported data from the previous study (Poché et al., 2011). Numbers of _P. argentipes_ collected per trap night were significantly higher than those of _Sergentomyia_ spp. (\(P = 0.0029\)). The highest
**Table 2.** Sex and heights of palmyra palm trees in which Centers for Disease Control light traps were set, and from which sandflies were collected, during September–December 2015 in the village of Sutihaar.

| Palm trap no. | Sex of tree | Height, m |
|---------------|-------------|-----------|
| P-1           | Male        | Unknown*  |
| P-2           | Female      | Unknown*  |
| P-3           | Female      | 11.8      |
| P-4           | Male        | 12.4      |
| P-5           | Female      | 12.8      |
| P-6           | Male        | 12.6      |
| P-7           | Male        | 12.2      |
| P-8           | Female      | 13.7      |
| P-9           | Male        | 12.1      |
| P-10          | Female      | 12.1      |
| P-11          | Female      | 10.9      |
| P-12          | Male        | 11.4      |

*Trees were burned in a village fire before measurements could be taken. Measurements were taken at the end of the study, after sandfly collection was completed.

A high proportion of sandflies (∼63%) were collected from areas in which bananas (*Musa acuminata*) were present. A total of 1113 sandflies, amounting to an average of 46.4 sandflies per trap night, were collected from two traps positioned in areas with banana plants (trap nos. GLE-1 and GLE-5). This was noticeably higher than the cumulative number of sandflies (*n* = 651) and mean number of sandflies per trap night (18.1) collected from the three traps positioned in other areas in which bananas were not present (trap nos. GLE-2, GLE-3, GLE-4) (Table 3). Additionally, the number of sandflies collected per trap night in locations with banana plants was significantly higher than numbers collected in other areas (*P* = 0.0141).

**Sutihaar: palmyra palm tree captures.** A total of 1786 sandflies were collected from palm trees over 144 trap nights to give an average of 1.6 *P. argentipes*, 10.8 *Sergentomyia* spp. and 12.4 numbers were captured on 2 December (*n* = 363) and 25 November (*n* = 309). The lowest number was captured on 23 September (*n* = 17).

**Fig. 3.** Mean numbers of sandflies collected per trap night (23 September to 9 December) in (A) five Centers for Disease Control (CDC) light traps positioned in outlying vegetation in the village of Mahesia (1259 *Phlebotomus argentipes* and 505 *Sergentomyia* spp.), and (B) 12 CDC light traps positioned in the canopies of 12 palmyra palm trees in the village of Sutihaar (231 *P. argentipes* and 1553 *Sergentomyia* spp.). Vertical bars represent the standard error. [Colour figure can be viewed at wileyonlinelibrary.com].
trees (flies were collected per trap night in female trees than in male and male palms, respectively (Table 4). Significantly more sandflies were collected from outlying vegetation containing banana plants and three CDC light traps set in other outlying vegetation.

The results of this study provide further evidence of consistent exophilic, nocturnal sandfly behaviour. As in previous experiments, in which over 7500 (Poché et al., 2011) and over 5000 (Poché et al., 2012) sandflies were collected from outlying vegetation in Mahesia and from palmyra palm trees in Sutihaar, respectively, sandflies were successfully collected from outlying vegetation and palmyra palm trees in the same two villages from September to December 2015.

Although the role of vegetation in determining sandfly distribution remains unclear, the present study suggests the relationship between sandflies and vegetation surrounding villages should be further evaluated. The current study reinforces the results of previous studies performed in the same area (Poché et al., 2011, 2012) and also provides a more explicit evaluation of the locations of adult sandfly captures in an attempt to determine any preference for specific vegetation within Bihari villages. Although only female sandflies feed on blood (because they require nutrients for egg production), both male and female sandflies feed on natural sources of sugar such as vegetation sap (Killick-Kendrick, 1999). Sugar-rich sap is produced by banana plants (Pothavorn et al., 2010) and palmyra palm trees (Barh & Mazumdar, 2008). In addition, according to (X. Chowdhury et al., unpublished data, 2016) collected in Nepal, sugary sap is produced.

### Discussion

The results of this study provide further evidence of consistent exophilic, nocturnal sandfly behaviour. As in previous experiments, in which over 7500 (Poché et al., 2011) and over 5000 (Poché et al., 2012) sandflies were collected from outlying vegetation in Mahesia and from palmyra palm trees in Sutihaar, respectively, sandflies were successfully collected from outlying vegetation and palmyra palm trees in the same two villages from September to December 2015.

Although the role of vegetation in determining sandfly distribution remains unclear, the present study suggests the relationship between sandflies and vegetation surrounding villages should be further evaluated. The current study reinforces the results of previous studies performed in the same area (Poché et al., 2011, 2012) and also provides a more explicit evaluation of the locations of adult sandfly captures in an attempt to determine any preference for specific vegetation within Bihari villages. Although only female sandflies feed on blood (because they require nutrients for egg production), both male and female sandflies feed on natural sources of sugar such as vegetation sap (Killick-Kendrick, 1999). Sugar-rich sap is produced by banana plants (Pothavorn et al., 2010) and palmyra palm trees (Barh & Mazumdar, 2008). In addition, according to (X. Chowdhury et al., unpublished data, 2016) collected in Nepal, sugary sap is produced.
high number of sandflies captured in banana plants may result from the nutritional quality of the sap produced. Additionally, organic material from banana plants and palm trees may provide substrate for *P. argentipes* oviposition. A comparison of collections by tree gender shows that a higher proportion of sandflies were collected from female palm trees (∼63%), a trend observed by Poché *et al.* (2012). The abundance of sandflies in female palm tree palms observed in the current study and previously by Poché *et al.* (2012) may be attributable to the excess pulp and mature fruit, and hence organic material, produced by female trees. Oviposition site surveys performed in Bihar and other parts of India focus largely on the collection of soil and organic material from in and around homes and cattle sheds (Ghosh & Bhattacharya, 1991; Kundu *et al.*, 1995; Singh *et al.*, 2008), historically yielding low numbers of sandflies. Outlying vegetation, including palm trees and banana plants, may provide sandflies with a variety of sources of organic material in which to oviposit. Although immature sandflies are rarely found during oviposition site surveys, in one study over 2000 sandfly larvae were recovered from forest floors in Panama (Hanson, 1961). Therefore, oviposition site surveys should not be limited to man-made dwellings but should additionally include a variety of organic matter types surrounding villages. Narrowing the sizable gap in knowledge of sandfly oviposition sites, and hence of the locations of wild immature sandflies, should be a chief concern amongst managers and ecologists alike.

Additionally, the ability of sandflies to move vertically calls into question the belief that they are poor flyers. All of the palm trees from which sandflies were collected during this study were over 10 m in height. In a study currently in progress in Bihar, India, researchers observed *P. argentipes*, dusted with fluorescent powder, dispersing vertically up to around 6 m before disappearing from sight (D. M. Poché, Z. Torres, G. Garlapati, R. Poché; ‘Monitoring the short-term movement of *Phlebotomus argentipes* in villages in Bihar, India’; unpublished study, 2016). This suggests that *P. argentipes* may be more capable of vertical and horizontal dispersal than previously suggested. Results of a previous mark–release–recapture study conducted by Killick-Kendrick *et al.* (1984) indicated extended lateral mobility in *Phlebotomus argentipes* in France, with the maximum distances travelled from the release point being 600 m and 2.2 km in males and females, respectively. Recently, dispersal experiments in Israel determined the maximum travel distances of wild *Phlebotomus papatasi* to be 1.51 and 1.91 km in males and females, respectively (Orshan *et al.*, 2016). If *P. argentipes* possesses this level of dispersal capability, contrary to the prevailing belief that sandflies do not disperse far from breeding sites (Munstermann, 2004), further questions regarding disease transmission and vector competence in Bihar must be investigated. Assuming that greater dispersal capability increases the capacity of *P. argentipes* to transmit VL to previously non-endemic areas, vector control at the village level may need to be re-evaluated. Hence, if the movement of *P. argentipes* is not limited to the microhabitats available in individual villages and *P. argentipes* proves to be capable of moving laterally between neighbouring villages, vector control programmes will need to take this into account and to consider attempting sandfly control on a larger scale.

Therefore, it is recommended that future research should focus on the dispersal capability of *P. argentipes*.

It is interesting to note that *Sergentomyia* spp. outnumbered *P. argentipes* in palm tree palms in the current experiment. Numbers of *Sergentomyia* spp. (*n* = 1553) were markedly higher than those of *P. argentipes* (*n* = 231), a trend opposite to that observed by Poché *et al.* (2012). However, a study of vertical distribution performed in Kenya reported *Sergentomyia bedfordi* and *Sergentomyia antennata* as the only sandfly species to fly up to 11 m above the ground (Basimike *et al.*, 1989). It would be useful in future studies to attempt to determine if specific geographic and/or climatic variability is responsible for the differences between current and previous results.

Conclusions

The results of the present study reinforce a need for integrated vector management incorporating novel control and preventative strategies to supplement the current practice of IRS. Perry *et al.* (2013) concluded that inhabitants in the majority of households within Bihari villages (∼95%) sleep outdoors for at least part of the year, particularly during the hot summer months. A study in which 93.7% of the 626 Bihari VL patients interviewed confirmed that they slept outdoors for part of the year drew similar conclusions (E. Hasker, R. Garlapati, R. Topno, A. Picado, M. Boelaert, R. Poché, P.K. Das; ‘Vector control for visceral leishmaniasis in India, are we targeting the right sandfly populations?’; unpublished study, 2016). Studies from India (Barnett *et al.*, 2005) and Nepal (Bern *et al.*, 2000) have concluded that sleeping outdoors is a significant risk factor for VL. Additionally, studies have found that bednet usage decreases in response to high evening temperatures (Kumar *et al.*, 2009; Claborn, 2010). Therefore, implementing an integrated vector control programme that combines a control strategy based on the resting sites of adult sandflies (IRS) with one dependent on the host and oviposition site preferences of sandflies (systemic insecticides) is encouraged. Previous studies from India involving *P. argentipes* (Ingenloff *et al.*, 2012; Poché *et al.* 2012) and Kenya involving *Anopheles* spp. (Diptera: Culicidae) mosquitoes (Poché *et al.*, 2015) have demonstrated the potential for the systemic insecticide fipronil to eliminate blood-feeding vectors feeding on cattle and rodents and larvae feeding on excreted faeces. Additionally, if advantageous data regarding oviposition sites in Bihar become available, explicit larval control should be administered. Hence, the results of the present study raise questions regarding sandfly dispersal, oviposition and feeding behaviours and suggest a need to refine current control practices in India in a manner that takes into account an evolving understanding of *P. argentipes* ecology.

Acknowledgement

The authors thank the Bill and Melinda Gates Foundation (grant no. 5112) for providing funding for the present research.
References

Alvar, J., Velez, I.D., Bern, C. et al. (2012) Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*, 7, e35671.

Barb, D. & Mazumdar, B.C. (2008) Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date (*Phoenix sylvestris Rosb.*) sap in treatment of anaemia. *Research Journal of Medicine and Medical Sciences*, 3, 173–176.

Barnett, P.G., Singh, S.P., Bern, C., Hightower, A.W. & Sundar, S. (2005) Virgin soil: the spread of visceral leishmaniasis into Uttar Pradesh, India. *American Journal of Tropical Medicine and Hygiene*, 73, 720–725.

Basimike, M., Mutinga, M.J. & Mutero, C.M. (1989) Vertical distribution of phlebotomine sandflies in two habitats in Marigat leishmaniasis endemic focus, Baringo district, Kenya. *International Journal of Tropical Insect Science*, 10, 645–650.

Bern, C., Joshi, A.B., Jha, S.N. et al. (2010) The biology and control of leishmaniasis vectors. *Tropical Medicine and International Health*, 14, 639–644.

Boelaert, M., Meheus, F., Sanchez, A. et al. (2009) The poorest of the poor: a poverty appraisal of households affected by visceral leishmaniasis in Bihar, India. *Tropical Medicine and International Health*, 14, 77–82.

Chappuis, F., Sundar, S., Hallu, A. et al. (2007) Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nature Reviews Microbiology*, 5, 873–882.

Coleman, M., Foster, G.M., Deb, R. et al. (2015) DDT-based indoor residual spraying suboptimal for visceral leishmaniasis elimination in India. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 8573–8578.

Desjeux, P. (2001a) The increase in risk factors for leishmaniasis worldwide. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 95, 239–243.

Desjeux, P. (2001b) Worldwide increasing risk factors for leishmaniasis. *Medical Microbiology and Immunology*, 190, 77–79.

Dinesh, D.S., Kar, S.K., Kishore, K. et al. (2000) Screening sandflies for natural infection with *Leishmania donovani*, using a non-radioactive probe based on the total DNA of the parasite. *Annals of Tropical Medicine and Parasitology*, 94, 447–451.

Garlapati, R.B., Abbasi, I., Warburg, A., Poché, D. & Poché, R. (2012) Identification of bloodmeals in wild caught blood fed *Phlebotomus argentipes* (Diptera: Psychodidae) using cytochrome b PCR and reverse line blotting in Bihar, India. *Journal of Medical Entomology*, 49, 515–521.

Ghosh, K.N. & Bhattacharya, A. (1991) Breeding places of *Phlebotomus argentipes* Annandale and Brunetti (Diptera: Psychodidae) in West Bengal, India. *Parasitologia*, 33, 267–272.

Hanson, W.J. (1961) The breeding places of *Phlebotomus* in Panama (Diptera, Psychodidae). *Annals of the Entomological Society of America*, 54, 317–322.

Ingenloff, K., Garlapati, R., Poché, D., Singh, M.I., Remmers, J.L. & Poché, R.M. (2012) Feed-through insecticides for the control of the sandfly *Phlebotomus argentipes*. *Medical and Veterinary Entomology*, 27, 10–18.

Killick-Kendrick, R. (1999) The biology and control of phlebotomine sandflies. *Clinics in Dermatology*, 17, 279–289.

Killick-Kendrick, R., Rioux, J.A., Bailly, M. et al. (1984) Ecology of leishmaniasis in the south of France. 20. Dispersal of *Phlebotomus ariasi* Tomnoir, 1921 as a factor in the spread of visceral leishmaniasis in the Cevennes. *Annales de Parasitologie Humaine et Comparée*, 59, 555–572.

Kumar, N., Siddiqui, N.A., Verma, R.B. & Das, P. (2009) Knowledge about sandflies in relation to public and domestic control activities of kala-azar in rural endemic areas of Bihar. *Journal of Communicable Diseases*, 41, 121–128.

Kundu, M., Basak, B. & Tandon, N. (1995) A simple technique for detection and isolation of *Phlebotomus argentipes* larvae from soil samples. *Journal of Communicable Diseases*, 27, 58–59.

Munstermann, L. (2004) Phlebotomine sandflies, the Psychodidae. *Biological Disease Vectors*, 2nd edn (ed. by W. Marquardt, W. Black, J. Freier, et al.), pp. 141–151. Elsevier, San Diego, CA.

Orshon, L., Elbaz, S., Ben-Ari, Y. et al. (2016) Distribution and dispersal of *Phlebotomus papatasi* (Diptera: Psychodidae) in a zoontic cutaneous leishmaniasis focus, the Northern Negev, Israel. *PLoS Neglected Tropical Diseases*, 10, e0004819.

Perry, D., Dixon, K., Garlapati, R., Genderalnik, A., Poché, D. & Poché, R. (2013) Visceral leishmaniasis prevalence and associated risk factors in the saran district of Bihar, India, from 2009 to July of 2011. *American Journal of Tropical Medicine and Hygiene*, 88, 778–784.

Poché, D., Garlapati, R., Ingenloff, K., Remmers, J. & Poché, R. (2011) Bionomics of phlebotomine sandflies from three villages in Bihar, India. *Journal of Vector Ecology*, 36 (Suppl.), 106–117.

Poché, R.M., Garlapati, R., Elinaem, D.E.A., Perry, D. & Poché, D. (2012) The role of palmyra palm trees (*Borassus flabellifer*) and sandfly distribution in northeastern India. *Journal of Vector Ecology*, 37, 148–153.

Poché, R.M., Burruss, D., Polyakova, L., Poché, D.M. & Garlapati, R.B. (2015) Treatment of livestock with systemic insecticides for control of *Anophelus arabiensis* in western Kenya. *Malaria Journal*, 14, 1–9.

Pothavorn, P., Kitdumrongson, K., Swangpol, S. et al. (2010) Sap phytochemical compositions of some bananas in Thailand. *Journal of Agricultural and Food Chemistry*, 58, 8762–8773.

Singh, R.K., Pandey, H.P. & Sundar, S. (2006) Visceral leishmaniasis (kala-azar): challenges ahead. *Indian Journal of Medical Research*, 123, 331–344.

Singh, R., Lal, S. & Saxena, V.K. (2008) Breeding ecology of visceral leishmaniasis vector sandfly in Bihar state of India. *Acta Tropica*, 107, 117–120.

Warburg, A. & Fairman, R. (2011) Research priorities for the control of phlebotomine sandflies. *Journal of Vector Ecology*, 36 (Suppl.), 10–16.

Accepted 28 November 2016
First published online 20 January 2017