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Short Communication

New Records and Updated Checklist of Phlebotomine Sand Flies (Diptera: Psychodidae) From Liberia

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Abstract

Phlebotomine sand flies from three counties in Liberia were collected from January 2011 to July 2013. In total, 3,118 sand flies were collected: 18 species were identified, 13 of which represented new records for Liberia. An updated taxonomic checklist is provided with a brief note on sand fly biology, and the disease vector potential for species is discussed.

Key words: Leishmaniasis, *Phlebotomus*, *Sergentomyia*, West Africa

Phlebotomine sand flies are fragile, nocturnally active insects with blood-feeding females that serve as important vectors of *Leishmania*, *Bartonella bacilliformes*, and a number of arboviruses across the Old and New World (Alexander 2000, Ready 2013). The sand fly fauna in Liberia has rarely been studied, and little is known about the role that certain species may play in the transmission of leishmaniasis and other diseases. Located along the West African coast, Liberia encompasses 110,000 km², bordering Sierra Leone to the north, Guinea to the northeast, and Côte D'Ivoire to the east. In the western forest belt of the Afrotropical Biotic Region, Liberia is characterized primarily by mangrove swamps along the coast, rolling hills, and dense tropical forests (Stojanovich and Scott 1966). According to Seccombe et al. (1993) and the Walter Reed Biosystematics Unit [WRBU] (2015 www.sandflycatalog.org), only five species of phlebotomines have been recorded from Liberia. Until this study, many more species of sand flies were suspected of existing in Liberia but had not been identified. As part of a larger mosquito surveillance study (Obenauer et al. 2013), we collected sand flies using various types of light traps and identified them to update sand fly records for the country.

Materials and Methods

Sand flies were surveyed from three counties in Liberia during 15 site visits from January 2011 to July 2013. Common flora throughout the sites included oil palms (*Elaeis guineensis* Jacquin), coconut

palms (*Cocos nucifera* L.), silk cotton (*Ceiba pentandra* L.), rubber trees (*Hevea brasiliensis* Müller Argoviensis), giant bamboo (*Bambusa oldhamii* Munro), and cassava (*Manihot esculenta* Crantz). Sand fly adults were collected using the Centers for Disease Control and Prevention (CDC) miniature light trap (model 512, John W. Hock Company, Gainesville, FL), the 4-W miniature ultraviolet (UV) CDC light trap (Bioquip Products, Rancho Dominguez, CA), and the UV light-emitting diodes (LED) light trap (Bioquip Products). Traps were suspended along a transect 20 m apart from each other and supplemented with attractants including the synthetic BG-Lure (Biogents, Germany) and carbon dioxide generated from fermented yeast (Obenauer et al. 2013) to improve catches. Sand fly specimens were frozen in a dry ice cooler for 1 h, enumerated, and placed into 1.5-ml plastic microcentrifuge tubes containing a 75% ethanol solution. Sand flies were slide-mounted, sexed, and identified to species using pertinent literature (Abonnenc and Minter 1965, Abonnenc 1972, Lewis 1982, Lane 1986, Davidson 1990). The DNA was extracted from the alimentary canals from *Phlebotomus* species using QIAGEN DNV Mini Kit (QIAGEN, Valencia, CA) and tested for *Leishmania tropica* using real-time polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism as previously described (Villinski et al. 2008).

Nomenclaturally, we use the abbreviations proposed by Marcondes (2007) for genera and subgenera of Phlebotominae. Voucher specimens of all species collected from Liberia were sent to the U.S. Army WRBU, Smithsonian Institution, Suitland, MD, for species confirmation and to permanently deposit in the museum. To

update the sand fly list, an extensive review of the literature was conducted for sand flies of Liberia using the WRBU catalog (www.sandflycatalog.org), VectorMap 2015 (www.vectormap.org), and the Armed Forces Pest Management Board Literature Retrieval Service (AFPMB-LRS 2015; http:afpmb.org/content/welcome-literature-retrieval-system).

Results and Discussion

In total, 3,118 sand flies representing 18 species in two genera (*Phlebotomus* and *Sergentomyia*) were collected in Lofa, Margibi, and Montserrado counties. From these collections, 13 species were determined to be new records for Liberia (Table 1). Table 2 gives an updated taxonomic checklist of phlebotomine species known from Liberia. Our survey yielded four species of genus *Phlebotomus* belonging to three subgenera: *Adlerius* (one sp.), *Anapaplebotomus* (one sp.), and *Paraplebotomus* (two spp.). Our collections of genus *Sergentomyia* comprised 14 species in three subgenera: *Grassomyia* (one sp.), *Neophlebotomus* (three species), *Parrotomyia* (three spp.), *Sergentomyia* (four spp.), and three ungrouped species. Some of our specimens had damaged or missing diagnostic body parts, so could not be identified to species level, including the following taxa: *Sergentomyia* (*Grassomyia*), one female; *Se.* (*Neophlebotomus*), two females; *Se.* (*Sergentomyia*), one male; and *Se.* (*ungrouped*), one male and one female.

From 2011–2013, the greater numbers of sand flies were collected during May–August (Fig. 1), exhibiting a major peak in May 2012. The coastal area of Liberia received an average of 462 cm of annual precipitation, the greatest amount occurring during the rainy season from May to September (Climatemps 2015). *Phlebotomus* (*Paraplebotomus*) *sergenti* Parrot, *Phlebotomus* (*Paraplebotomus*) *kazeruni* Theodor & Mesghali, and *Phlebotomus* (*Adlerius*) *arabicus* Theodor were only collected from Montserrado County. *Sergentomyia* (*Grassomyia*) *squamipleuris* Newstead, a new species for Liberia, was the predominant species of sand fly collected, comprising over 30% of the catch (Table 1). In Africa, similar

Table 1. Phlebotomine sand flies collected from three counties of Liberia from January 2011 to July 2013

Species	Counties	Total (%)
<i>Sergentomyia squamipleuris</i> Newstead ^a	Lofa, Margibi	951 (30.5)
<i>Se. africana</i> Newstead ^a	Lofa	454 (14.6)
<i>Se. schwetzi</i> Adler, Theodor, Parrot	Lofa	373 (12.0)
<i>Se. dureni</i> Parrott	Lofa	338 (10.8)
<i>Se. simillima</i> Newstead ^a	Lofa	288 (<0.1)
<i>Se. bedfordi</i> Newstead ^a	Lofa	213 (<0.1)
<i>Se. collarti</i> Adler, Theodor, and Parrot ^a	Lofa	129 (<0.1)
<i>Se. dissimillima</i> Abonnenc ^a	Lofa	118 (<0.1)
<i>Se. crosarai</i> Parrot and Wanson ^a	Lofa, Margibi	108 (<0.1)
<i>Se. impudica</i> Abonnenc	Lofa	84 (<0.1)
<i>Phlebotomus rodhaini</i> Parrot	Lofa	23 (<0.1)
<i>Se. ingrami</i> Newstead ^a	Lofa	17 (<0.1)
<i>Se. eremitis</i> Parrot & Bouquet de Joliniere ^a	Lofa	8 (<0.1)
<i>Se. moreli</i> Abonnenc and Hamon ^a	Lofa	6 (<0.1)
<i>Se. antennata</i> Newstead	Lofa, Margibi	4 (<0.1)
<i>Ph. sergenti</i> Parrot ^a	Montserrado	2 (<0.1)
<i>Ph. kazeruni</i> Theodor & Mesghali ^a	Montserrado	1 (<0.1)
<i>Ph. arabicus</i> Theodor ^a	Montserrado	1 (<0.1)

^a New country record.

Species listed in descending order, based on the total numbers of each species collected.

observations were made by Lewis (1971), who noted that light traps caught high numbers of *Se. squamipleuris* compared with other sand fly species. Although not known to be disease vectors, *Sergentomyia* (*Sergentomyia*) *schwetzi* Adler, Theodor, and Parrot and *Sergentomyia* (*Parrotomyia*) *africana* Newstead are anthropophilic (Lewis 1971, Lewis and Murphy 1965). Both species were collected in large numbers, comprising 26% of the catch (Table 1). A well-known exophilic species, *Se. schwetzi*, has been collected predominantly from many peridomestic habitats throughout West Africa, especially communities with many dogs (Anderson et al. 2011, Senghor et al. 2011).

Leishmaniasis is considered endemic in West Africa, with reported cases in Mali, Nigeria, Senegal, Chad, Mauritania, Gambia, and Ghana (Boakye et al. 2005, Anderson et al. 2011). According to Cahill (1968), several intensive studies conducted between 1910 and 1950 determined that cutaneous leishmaniasis was limited to arid regions between 10 and 20 degrees north latitude, while visceral leishmaniasis was focused in Gabon, Democratic Republic of the Congo, Angola, and Nigeria. Human cases of dermal leishmaniasis in Ho District of Ghana have recently been attributed to the

Table 2. Updated checklist of Phlebotomine sand fly species from Liberia

Species	Reference
<i>Phlebotomus</i> (<i>Adlerius</i>) <i>arabicus</i> Theodor, 1953 ^a	X ^b
<i>Phlebotomus</i> (<i>Anaplebotomus</i>) <i>rodhaini</i> Parrot, 1930	S, W, X
<i>Phlebotomus</i> (<i>Paraplebotomus</i>) <i>kazeruni</i> Theodor and Mesghali, 1964 ^a	X
<i>Phlebotomus</i> (<i>Paraplebotomus</i>) <i>sergenti</i> Parrot, 1917 ^a	X
<i>Sergentomyia</i> (<i>Grassomyia</i>) <i>squamipleuris</i> Newstead, 1912 ^a	X
<i>Sergentomyia</i> (<i>Neophlebotomus</i>) <i>collarti</i> Adler, Theodor, and Parrot, 1929 ^a	X
<i>Sergentomyia</i> (<i>Neophlebotomus</i>) <i>dureni</i> Parrot, 1934	S, W, X
<i>Sergentomyia</i> (<i>Neophlebotomus</i>) <i>ingrami</i> Newstead, 1914 ^a	X
<i>Sergentomyia</i> (<i>Parrotomyia</i>) <i>africana</i> Newstead, 1912 ^a	X
<i>Sergentomyia</i> (<i>Parrotomyia</i>) <i>crosarai</i> Parrot & Wanson, 1946 ^a	X
<i>Sergentomyia</i> (<i>Parrotomyia</i>) <i>eremitis</i> Parrot & Bouquet de Joliniere, 1945 ^a	X
<i>Sergentomyia</i> (<i>Sergentomyia</i>) <i>antennata</i> Newstead, 1912	S, W, X
<i>Sergentomyia</i> (<i>Sergentomyia</i>) <i>bedfordi</i> Newstead, 1914 ^a	X
<i>Sergentomyia</i> (<i>Sergentomyia</i>) <i>impudica</i> Abonnenc, 1968	S, W, X
<i>Sergentomyia</i> (<i>Sergentomyia</i>) <i>schwetzi</i> Adler, Theodor, and Parrot, 1929	S, W, X
<i>Sergentomyia</i> (<i>Ungrouped</i>) <i>dissimillima</i> Abonnenc, 1972 ^a	X
<i>Sergentomyia</i> (<i>Ungrouped</i>) <i>moreli</i> Abonnenc & Hamon, 1958 ^a	X
<i>Sergentomyia</i> (<i>Ungrouped</i>) <i>simillima</i> Newstead, 1914 ^a	X

^a New species record for Liberia (= 13 out of 18 total reported species).

^b S (Seccombe et al. 1993), W (WRBU 2015), and X (this survey).

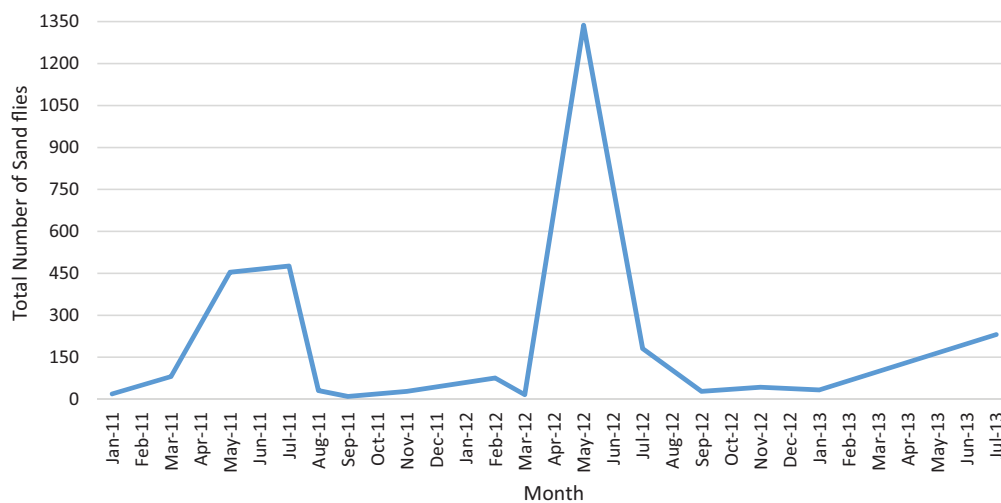


Fig. 1. Seasonal abundance of Phlebotomine sand flies collected from three counties of Liberia (January 2011–July 2013).

Leishmania enriettii complex (Kwakye-Nuako et al. 2015), but we found no reports of any leishmaniasis from Liberia.

Of the 18 sand fly species collected in our study in Liberia, two species *Pb. (Adl.) arabicus* and *Pb. (Par.) sergenti* have been implicated as vectors of *Leishmania* parasites in Africa (Ready 2013). *Phlebotomus sergenti* represents a proven vector for anthroponotic cutaneous leishmaniasis (ACL). However, *Leishmania (Leishmania) tropica* (Wright), the causative organism for ACL, is focused in drier parts of West Africa and transmitted primarily by *Phlebotomus (Pbl.) duboscqi* Neveu-Lemaire (Killick-Kendrick 1999). In his review of the Phlebotomine sand flies, Ready (2013) noted that *Pb. sergenti*, *Pb. arabicus*, and *Phlebotomus (Lar.) guggisbergi* Kirk and Lewis are proven vectors of *Le. tropica* in Africa. A new record for Liberia *Pb. sergenti* was collected on the outskirts of downtown Monrovia (Montserrado County), which is not unexpected because of its reported propensity for peridomestic habitats and human hosts (Ready 2013). Two *Pb. sergenti* specimens in our collections tested negative for the presence of *Le. tropica*; however, it could be a potential vector for ACL and future investigations are warranted to determine its vector status in Liberia. Moreover, in Senegal, Senghor et al. (2011) hypothesized that *Se. schwetzi* might be a potential vector of canine leishmaniasis, challenging the dogma that only *Phlebotomus* species are capable of transmitting *Leishmania* in the Old World. *Sergentomyia (Sergentomyia) bedfordi* Newstead and *Se. (Par.) africana* are also new records for Liberia, and while not reported disease vectors, they are worth mentioning for consistently biting humans in Kenya and Sudan, respectively (Lewis and Murphy 1965, Seccombe et al. 1993).

While we did not use a variety of trapping methods to elucidate sand fly microhabitats such as tree holes or vegetation types, surveillance conducted in the Gambia demonstrated that *Se. squamipleuris* is typically collected by light traps in areas with permanently moist soil (Lewis and Murphy 1965). Additionally, their study showed that more sand fly species were collected in fields of oil palms and rice than in other habitats. Similarly, the majority of our collected sand flies were from sites that were adjacent to rice field or savannah-type environments. We only surveyed sand flies in three counties of Liberia, and more comprehensive sand fly surveillance is warranted from the remaining 11 counties to provide a better assessment of the sand fly fauna throughout the country. In addition, future collections should use different sampling methods to collect a possibly wider

range of species, and to determine anthropophilic versus zoophilic behavior and exophily versus endophily, for inferring risks of *Leishmania* pathogen transmission to the people of Liberia.

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References Cited

- Abonnenc, E. 1972. Les Phlebotomes de la region Ethiopienne (Diptera, Psychodidae). Memoires Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), No. 55: 1–289.
- Abonnenc, E., and D. M. Minter. 1965. Keys for the identification of sandflies of the Ethiopian region. Cahiers Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), Ser. Entomol. Med. 5: 24–63.
- Alexander, B. 2000. Sampling methods for phlebotomine sandflies. Med. Vet. Entomol. 14: 109–122.
- Anderson, J. M., S. Samake, G. Jaramillo-Gutierrez, I. Sissoko, C. A. Coulibaly, B. Traoré, C. Souko, B. Guindo, D. Diarra, M. P. Fay, et al. 2011. Seasonality and prevalence of *Leishmania major* infection in *Phlebotomus duboscqi* Neveu-Lemaire from two neighboring villages in central Mali. PLoS Negl. Trop. Dis. 5: e1139.

- AFPMB-LRS. 2015. Armed forces pest management board literature retrieval system (AFPMB-LRS). (http://afpmb.org/content/welcome_literature-retrieval-system) (accessed 22 October 2015).
- Boakye, D. A., M. D. Wilson, and M. Kweku. 2005. A review of leishmaniasis in west Africa. *Ghana Med. J.* 39: 94–97.
- Cahill, K. M. 1968. Clinical and epidemiological patterns of leishmaniasis in Africa. *Trop. Geogr. Med.* 20: 109–118.
- Climatemps. 2015. (<http://www.liberia.climatemps.com>)
- Davidson, I. H. 1990. Sandflies of Africa South of the Sahara: Taxonomy and systematics of the genus *Sergentomyia*, p. 35. Department of Medical Entomology, South African Institute for Medical Research, Johannesburg, South Africa.
- Killick-Kendrick, R. 1999. The biology and control of Phlebotomine sand flies. *Clin. Dermatol.* 17: 279–289.
- Kwakye-Nuako, G., M. T. Mosore, C. Duplessis, M. D. Bates, N. Pupilampu, I. Mensah-Attipoe, K. Desewu, G. Afegbe, R. H. Asmah, M. B. Jamjoom, et al. 2015. First isolation of a new species of *Leishmania* responsible for human cutaneous leishmaniasis in Ghana and classification in the *Leishmania enriettii* complex. *Int. J. Parasitol.* 45: 679–684.
- Lane, R. P. 1986. The sandflies of Egypt (Diptera: Psychodidae). *Bull. Br. Mus. Natl. Hist.* 52: 286–291.
- Lewis, D. J., and D. H. Murphy. 1965. The sand-flies of the Gambia (Diptera: Phlebotominae). *J. Med. Entomol.* 1: 371–376.
- Lewis, D. J. 1971. Phlebotomid sandflies. *Bull. World Health Org.* 44: 535–551.
- Lewis, D. J. 1982. A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bull. Br. Mus. Nat. Hist.* 45: 121–209.
- Marcondes, C. B. 2007. A proposal of generic and subgeneric abbreviations for Phlebotominae sandflies (Diptera: Psychodidae: Phlebotominae) of the world. *Entomol. News* 118: 351–356.
- Obenauer, P. J., M. S. Abdel-Dayem, C. A. Stoops, J. T. Villinski, R. Tageldin, N. T. Fahmy, J. W. Diclaro II, and F. Bolay. 2013. Field responses of *Anopheles gambiae* complex (Diptera: Culicidae) in Liberia using yeast-generated carbon dioxide and synthetic lure baited light traps. *J. Med. Entomol.* 50: 863–870.
- Ready, P. D. 2013. Biology of Phlebotomine sand flies as vectors of disease agents. *Annu. Rev. Entomol.* 58: 227–250.
- Secombe, A. K., P. D. Ready, and L. M. Huddleston. 1993. A catalogue of old world Phlebotomine sandflies (Diptera: Psychodidae, Phlebotominae) (Occasional Papers on Systematic Entomology). Intercept Ltd. Hampshire, UK.
- Senghor, M. W., M. N. Faye, B. Faye, K. Diarra, E. Elguero, O. Gaye, A. Bañuls, and A. A. Niang. 2011. Ecology of phlebotomine sand flies in the rural community of Mont Rolland (Thiès Region, Senegal): Area of transmission of canine leishmaniasis. *PLoS ONE* 6: e14773.
- Stojanovich, C. J., and H. G. Scott. 1966. Illustrated key to Anopheles mosquitoes of Liberia, p. 38. U.S. Department of Health Education and Welfare Public Health Service, Atlanta, GA.
- VectorMap. 2015. VectorMap, walter reed biosystematics unit. Smithsonian Institution, Museum Support Center, Suitland, MD. (<http://www.vectormap.org/>) (accessed 22 October 2015).
- Villinski, J. T., J. D. Klena, M. Abbassy, D. F. Hoel, N. Pupilampu, D. M. Boakye, and G. Racznik. 2008. Evidence for a new species of *Leishmania* associated with focal disease outbreak in Ghana. *Diagn. Microbiol. Infect. Dis.* 60: 323–268.
- (WRBU) Walter Reed Biosystematics Unit. 2015. Catalog of Subfamily Phlebotominae (Diptera: Psychodidae). Smithsonian Institution, Museum Support Center, Suitland, MD. (<http://www.sandflycatalog.org/>) accessed 22 October 2015).