New State Records for *Lutzomyia shannoni* and *Lutzomyia vexator*

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ABSTRACT Two species of phlebotomine sand flies, *Lutzomyia shannoni* (Dyar) and *Lutzomyia vexator* (Coquillett), are reported for the first time from Kentucky and Ohio. *L. vexator* also is reported for the first time from Tennessee. These insects were found in a northeasterly band extending from southwestern Kentucky to southwestern Ohio. Both species were consistently captured from mid-July through September in 2006 and 2007 by using CO_2-baited Center for Disease Control light traps. Weekly sampling revealed that these flies are more abundant in the southern part of this band than in the northern part, but increasing densities throughout this new range indicate that the flies are currently expanding their range. Although both species have been reported further north along the Atlantic coast, and *L. vexator* along the Pacific coast, neither of them had been reported this far north along the Mississippi Valley. Previous reports established *L. shannoni* as far north as west central Tennessee and *L. vexator* in a similar spatial pattern in the eastern part of its range, extending as far north as northern Alabama. Whether the new records reported herein represent a northerly expansion of the geographic range of these species or are reflective of sampling changes is inconclusive. However, the former scenario could presage an increased prevalence of the diseases associated with this group of insects.

KEY WORDS leishmaniasis, vesicular stomatitis virus, phlebotomine

Sand flies are small blood-feeding phlebotomines, typically occurring in the tropics and subtropics, and are primary vectors of several pathogens, including *Leishmania* spp. parasites, the causative agents of leishmaniasis. In the New World, the geographic range of the genus *Lutzomyia* extends as far south as Argentina, and northward through Mexico, the coastal areas of the United States, and into southern Canada (Young and Perkins 1984).

Recently, there has been a renewed interest in sand flies and their respective diseases due, in part, to American military involvement in the Middle East where leishmaniasis is endemic. However, domestic concerns also have heightened in the last few years. In particular, physicians in northern Texas reported nine human cases of locally acquired cutaneous leishmaniasis in 2006 and 2007. These cases occurred nearly 1,000 km north of the previously reported northern limit of the endemic range for this disease (Wright et al. 2008). The dermatologist who diagnosed one of the first cases and recognized their abnormality (Wright et al. 2008) speculated that these cases probably indicate a northerly expansion of the pathogen's range caused by a movement in the populations of either the reservoir or the vector.

A northward expansion of sand fly range would be a development of considerable interest to the public health and entomological communities. Kentucky and Ohio are two states that have not previously reported sand flies but are near the northerly limit of several common species. In this article, we report on the sand flies captured in these states and establish new state records for two species.

Materials and Methods

Sand fly populations were monitored over a 3-yr period (2005–2007). The first year, sand fly monitoring was conducted by the Uniformed Services University of the Health Sciences and was part of a larger effort to monitor flies at several army installations. The monitoring was more intensive (more sites and more sample dates) and included a larger geographical area in the second and third years.

Abundance and Seasonal Activity. Sand fly surveillance was conducted using standard Center for Disease Control (CDC) light traps (model 512, John W. Hancock, Gainesville, FL). The traps were all identical and were baited with light and ≈2.3 kg of dry ice (CO_2) pellets contained in 1.89-liter coolers (Con-

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tour, Igloo Products Corp., Houston, TX). The CO_2 gas was emitted via the spout on the top of the cooler and a through a 135-cm-long by 1-cm-diameter piece of vinyl tubing running from the bottom of the bottom of the cooler to near the light source. Two ≈1-cm holes also had been bored into the side of the cooler to allow CO_2 dispersal. The light source was generated from a 1.5-V incandescent light bulb located in its normal position, just above the fan. Each trap was powered by a 6.0-V rechargeable deep-well battery and suspended at a standard height of 1 m between the trap opening and the ground from identical metal hooks.

The traps were identical to the familiar mosquito traps except that the collection cups were lined 0.5-mm wire mesh. This is a finer mesh than that found in a standard mosquito trap and is necessary because adults in the genus *Lutzomyia* are smaller than most common mosquitoes (Young and Perkins 1984). When retrieving the traps, the sample cup was labeled, removed, and placed on dry ice to anesthetize the insects. The rest of the trap was then disassembled in reverse manner of setup. Coolers were noted to always contain at least some residual dry ice at the time of collection.

At Fort Campbell, surveillance trapping was conducted in September 2005 at a few sites. Weekly trapping was conducted over a 12-wk period (20 June–15 September) in 2006 and an 18-wk period in 2007 (20 June–19 October, omitting weeks of 22 July 2007 and 5 October 2007). The location for each site was selected with assistance from the U.S. Army 61st Preventive Medicine Detachment personnel based on land area coverage, vegetation, troop activity, and accessibility. The trap sites were generally placed among the highest density of deciduous forest easily accessible based on previous studies in which *Lutzomyia* spp. have been found to occur in such sites (Hanson 1961, Comer et al. 1993, Young and Duncan 1994). Location was recorded using a global positioning system receiver (Magellan III) and stored in standard Universal Transverse Mercator format. All future collections were taken from these specific locations.

There were a total of 10 trap sites selected, which were distributed over a nearly 140-km route, approximately encircling the Ft. Campbell U.S. Army base. The shortest distance between any two sites was ≈6.4 km, and the longest distance was 24 km. The closest trap site to the main base gate (gate 6) was designated trap site 1, and the trap sites were sequentially numbered from this initial position. Traps were set between 1600 and 1930 hours and retrieved the following morning between 0600 and 0900 hours. This covers the optimal period to attract phlebomotines that exhibit crepuscular feeding behavior (Young and Duncan 1994). During the 2007 season, the same trapping procedure was implemented, and the same locations were used.

After retrieval of all traps, the specimens were transported to the Public Health Entomology laboratory at the University of Kentucky and stored in a −15°C freezer. At the laboratory, all recovered sand flies were stored in 0.5-ml labeled vials, containing 70% ethanol.

All collected phlebotomines were cleared in a lactic acid-phenol based clearing solution (Bioquip Inc., Rancho Dominguez, CA). Upon clearing, the flies were temporarily mounted on glass microscope slides and identified under a compound light microscope at 20× magnification by using the standard North American phlebotomine key (Young and Perkins 1984). Voucher specimens have been deposited in the University of Kentucky Entomological Museum, Lexington, KY.

Additional Detections. In addition to studies at the Fort Campbell military installation, additional detection surveys were conducted where potential interactions between these insects and the general public could occur, namely, in areas used for agricultural and recreational activities. The first of these additional sites was selected in response to a report of a single sand fly specimen in 2006 (B.K., personal observation), near Lexington, KY. Traps identical to those used at Fort Campbell were set at various locations around the University of Kentucky's Spindletop Research Farm (Entomology section), which is located ≈25 km north of Lexington. Sites were chosen based on visual similarity to those sites at Fort Campbell that produce the most specimens. Sites were casually monitored from 21 July 2007 through 20 November 2007.

Similar detection surveys were conducted at two state parks and three farms in southwestern Ohio as well as a wildlife area in western Kentucky and two research farms in central Kentucky. For these locations, all sites selected were edge habitats, bordering upland, dry forested sites dominated by mature oak (*Quercus* spp.) trees.

Results and Discussion

Abundance and Activity. Three hundred and thirty three sand flies were recovered during sampling at Fort Campbell in 2006. During the 2007 season, 978 sand flies were captured at the same locations. During 2005, trapping occurred only from 6 to 8 September at 14 to 16 different sites and recovered a total of 155 sand flies. Based on the observations presented below for 2007, as well as observations in 2006 and 2008 (unpublished data), this seems to coincide with the peak activity of sand fly populations in this region. Therefore, to account for the differences in the duration of the study and number of traps set per night, comparisons between these years were conducted by comparing the average catch per trap per night in 2005 to the observed, average rates for 2006 and 2007 for the same time of year.

The average catch rate in 2005 was ≈3.4 sand flies per trap per night. In 2006, the value nearly doubled to 5.3 sand flies per trap night, and then again in 2007, to 10.5 sand flies per trap night. Thus, the average number of sand flies captured per trap night increased greatly over the 3 yr of observation at Fort Campbell. All specimens were identified to be *L. shannoni* and *L. vexator*. The data from the 2007 trap season were used

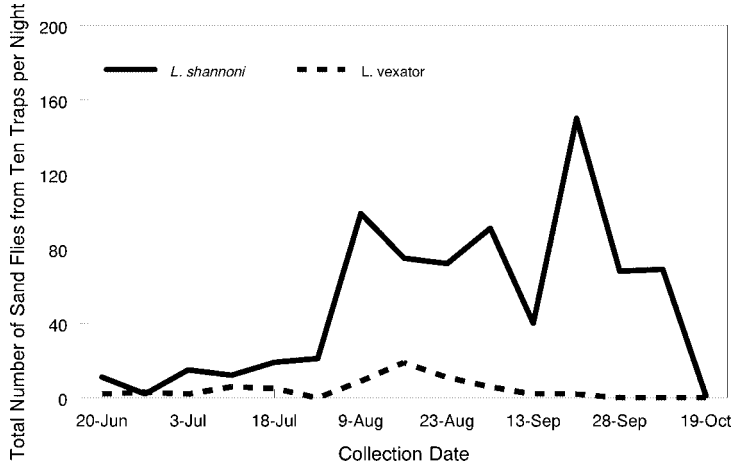

Fig. 1. Seasonal activity of *L. shannoni* and *L. vexator* at Fort Campbell, Kentucky, 2007.

to generate Fig. 1 that displays the catch total for each week at Fort Campbell. The numbers of *L. shannoni* captured remained relatively low and stable until mid-August when a large increase in trap catch was observed. The sand fly populations remained at this higher level through late September. This pattern indicates that two generations of adult *L. shannoni* occur in Kentucky per season.

It should be noted that although trapping was conducted on 31 August, essentially no flies were captured due to strong overnight thunderstorms. The results of this trapping period are not included here. Furthermore, no samples were taken during the week of 22 July (due to equipment malfunction) and during the week of 7 October, because afternoon temperatures were <10°C (flight threshold temperature for most Diptera). Previous studies have noted the absence of sand flies in CDC traps set when temperatures drop below this threshold (Brinson et al. 1992). Because of the low numbers captured throughout the season and their scattered distribution across sites, nothing further can be conclusively said of the seasonal activity of *L. vexator*. This pattern of low capture-rate was also true in the other years of sampling.

Additional Detections. As a result of the additional detection surveys conducted in central Kentucky and southwestern Ohio, four new county records for *L. shannoni* were established (Table 1). The most northern of these detections (Ross Co., OH) was ≈650 km northwest of Lake Co., TN, the previously most northern, inland detection site of *L. shannoni* (Young and Perkins 1984). To date, this marks the most northern record of this species west of the Appalachian Mountains.

In all, these captures (along with those mentioned previously from Fort Campbell) represent new state collection records for *L. shannoni* in Kentucky (Christian, Trigg, and Fayette counties) and Ohio (Ross, Pike, and Clermont counties), along with two additional county records in Tennessee (Montgomery and Stewart counties). The new records for *L. vexator* reported here are limited to the four counties comprising the Fort Campbell Installation (Christian and Trigg counties in Kentucky, and Montgomery and Stewart counties in Tennessee).

Discussion

Several public health concerns exist if the range of anthropophillic phlebotomine sand flies is moving northward into the heartland of the United States. Although *L. vexator*, which feeds on cold blooded vertebrates (Young and Perkins 1984), does not likely present a concern to public health, *Lutzomyia shannoni*, has been documented as a competent vector of a number of human and livestock diseases, including the New Jersey strain of vesicular stomatitis virus (Comer et al. 1990) and *Leishmania mexicana*, American Cutaneous leishmaniasis (Lawyer and Young 1987, Lawyer et al. 1987).

Additionally, a large number of canine visceral leishmaniasis cases across 21 states have been reported (Duprey et al. 2006). It has been reported that currently, most of the transmission is conducted through dog-to-dog interactions (Duprey et al. 2006). In the

Table 1. Locations, dates, and catch rates for additional sand fly trapping attempts in Kentucky and southwestern Ohio

Location	Date	Total	Catch rate[a]
UK North Farm, Fayette Co., KY	Summer 2007	71	2.44
Paint Creek State Park, Ross Co., OH	11 Aug. 2007	1	0.5
East Fork State Park, Clermont Co., OH	1 Sept. 2007	2	1
Prussia Ridge Farms, Pike/ Ross Co., OH	2 Sept. 2007	10	1.67
UK South Farm, Fayette Co., KY	6 Sept. 2007	0	0
Peabody Wildlife Area, Ohio Co., KY	25 Sept. 2007	0	0
UK Pin Oak Farm, Woodford Co., KY	4 Oct. 2007	0	0

[a] Catch rate indicates the number of sand flies captured per trap night, because not all detection traps were positive for sand flies.

same report, it was suggested that cases could increase if populations of infected dogs expand into the range of sand flies. However, no suggestion has been made that cases may increase if the opposite occurs and sand flies expand further into the range of infected dogs.

Several hypotheses could be formulated as to why these insects have not previously been reported in these areas, including: changes in weather or climatic patterns have allowed expansion and persistence of these species, alterations of landscapes have enhanced suitable habitats, the adaptation and development of a new biotype which is able to survive more northern climates, or that the insects are indigenous and have eluded past detection by investigators. At the time of publication, we have insufficient data to support any of these hypotheses and refrain from further speculation.

What is not speculative is that this paper documents an expansion of the known range of sand flies in central North America. Several populations of these flies have been detected outside of their historically known range, including Kentucky and Ohio, where *Leishmania*-infected dogs have been identified (Duprey et al. 2006). Although these insects have not been shown (in nature or in the laboratory) to be competent vectors of this particular strain of *Leishmania*, the possibility of future transmission of this disease to humans must not be prematurely discounted as a potential public health risk.

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