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PHLEBOTOMINE SAND FLIES IN LOUISIANA (DIPTERA:PSYCHODIDAE)¹

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Nine species and one subspecies of *Lutzomyia* (split off from the original genus *Phlebotomus* by Theodor, 1932, 1965) are known to occur in the United States, namely, *L. anthophora* (Addis), *L. aquilonia* (Fairchild and Harwood), *L. californica* (Fairchild and Hertig), *L. cruciata* (Coquillett) (= *P. diabolicus* Hall), *L. oppidana* (Dampf), *L. shannoni* (Dyar), *L. stewarti* (Mangabeira and Galindo), *L. texana* (Dampf), *L. vexatrix vexatrix* (Coquillett) (= *P. vexator* Coq.), and *L. vexatrix occidentis* (Fairchild and Hertig).

The only previous report of the occurrence of phlebotomine sand flies in Louisiana is that of Hall (1936), based on two specimens collected on May 25, 1935, by the Civilian Conservation Corps (CCC) Mosquito Survey in a light trap at Ansley, Jackson Parish, in the northern part of the State. The species was identified by

Dr. Alan Stone as *Phlebotomus vexator* Coquillett.

METHODS

A systematic search-and-survey was conducted in 1967 and 1968 to determine the presence, identity, distribution, and habits of phlebotomine sand flies in Louisiana. They were sought during daylight hours in natural cavities and hollow interiors of standing trees by inspection with a flashlight and injection of puffs of cigarette smoke or insect repellent spray (deet aerosol from a pressure can) to activate them. (While the spray penetrated hiding places more efficiently and was better for initial detection, it stimulated faster flight than smoke and was less suitable for disturbing the flies to facilitate capture. The flies are recognizable grossly by their small size, form, and characteristic erratic mode of flight.) They were collected by means of a simple aspirator consisting of a glass or transparent plastic tube, 0.5 inch in diameter and 10 to 12 inches long, to which a rubber tube 20 inches long was attached, with a screen barrier in the junction. The live insects were blown into a slightly moistened 4-oz. plaster-lined jar or earthenware pot that was provided with a cloth cover having a central aperture in a

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double layer of adhesive tape, closed with a cork stopper. The accumulated flies were released into a small sleeveage of netting and thence transferred by means of the aspirator to individual moist-plaster-lined, net-capped vials for transportation to the laboratory. These techniques and the methods used for rearing, dissecting, and mounting were developed by investigators at the Gorgas Memorial Laboratory in Panama during the past 20 years (Fairchild and Hertig, 1948; Hertig and Johnson, 1961). Only a few unsuccessful attempts were made to collect flies at night by means of a Shannon light trap, consisting of a suspended, hooded sheet of muslin illuminated by a gasoline lantern. Larvae were sought by the flotation and isolation methods described by Hanson (1961) and Thatcher (1968). Adults were identified by the study of dissected and mounted specimens.

The following keys, not previously available, to the males and females of species reported from the United States indicate their recognition characters, discernible only in dissected, slide-mounted specimens. The authors wish to express their sincere appreciation to Dr. G. B. Fairchild, Gorgas Memorial Laboratory, Panama, who kindly reviewed them and suggested modifications to insure their serviceability.

Keys to the Species of *Lutzomyia* (Franca) [*Phlebotomus* Rondani and Berté, pars] Known to Occur in the United States

Males

- 1. Style with three spines..... 2
 - Style with more than three spines.... 3
- 2. Style with a subterminal seta; coxite with two separate groups of setae at base; parameres with a small dorsal finger-like projection and a ventral triangular process; lateral lobes moderately inflated*L. aquilonia*
- Style without subterminal seta; coxite without basal setae; parameres with large, knob-like dorso-basal structure bearing long hooked hairs; lateral lobes not inflated, longer than paramere or coxite.....*L. anthophora*

- 3. Style with 4 spines; coxite with or without a tuft of hairs..... 4
 - Style with 5 spines; coxite with a tuft of hairs 6
- 4. Coxite with a medium tuft of 8 to 25 hairs or setae...*L. cruciata* (= *diabolica*)
 - Coxite without a median tuft of hairs or setae 5
- 5. Ascoids with a long proximal prolongation. Lateral lobes shorter than or equal to coxite. Palpal segments I and II equal in length to III and IV*L. shannoni*
 - Ascoids with a short proximal prolongation or none. Lateral lobes definitely longer than coxite. Palpal segments I and II equal in length to III*L. texana*
- 6. Basal spines on style inserted at markedly different distances from the base
 - Two basal spines on style inserted at or near same level..... 9
- 7. Genital filaments over 6 times as long as genital pump, tips slenderly spear-shaped; parameres slender, upcurved, slightly clubbed, bearing setae only on apical third.....*L. oppidana*
 - Genital filaments less than 5 times length of pump; parameres shorter, not upcurved, bearing dorsal setae nearly throughout their length..... 8
- 8. Genital filaments at least 4.5 times as long as pump; style long and cylindrical, all but the basal spine inserted well distal of its middle.....
 -*L. vexatrix vexatrix*
 - Genital filaments less than 4 times as long as pump; style shorter and stouter, the sub-basal spine inserted close to its middle...*L. vexatrix occidentis*
- 9. Basal tuft of coxite consisting of less than 5 setae; lateral lobes clearly longer than coxite.....*L. stewarti*
 - Basal tuft of coxite consisting of about a dozen stiff setae closely set on a small eminence; inner aspect of remainder of coxite with but a few rather long setae; lateral lobes subequal to coxite or shorter...*L. californica*

Females

- 1. Cibarium with a comb of about 18 to 20 teeth 2
 - Cibarium with 4 to 12 more or less triangular teeth 3
- 2. Cibarium with 18 horizontal teeth and numerous stout erect teeth. Sper-

- matheca spherical with a diameter about 2.5 times that of the duct, the ducts opening separately into the vagina; terminal knob of spermatheca small and discrete....*L. texana*
- Cibarium with 18 to 20 fine horizontal teeth, no erect teeth, a broad pigment patch and a weak sclerotized arch. Pharynx with a few flattened spines. Spermatheca elongate, with small rounded head and finely annulated body, about 1/3 the length of its duct; the 2 ducts joined to form a short common duct (about 1/16 of individual ducts) at the vagina*L. californica*
3. Spermatheca with numerous bubble-like expansions and the long terminal knob deeply invaginated into the body. Cibarium with about 4 blunt teeth, strong lateral teeth and a distinct strong sclerotized arch. 5th palpal segment the longest.....*L. anthophora*
- Spermatheca otherwise, without bubble-like expansions 4
4. Ascoids with long posterior prolongations. Cibarium with 4 horizontal pointed teeth, numerous small erect teeth and a strong chitinous arch. Spermathecae smooth, sausage shaped, with short individual ducts and long common duct....*L. shannoni*
- Ascoids without posterior prolongations. Spermathecae not as above.. 5
5. Cibarium with 2 broad rounded teeth and numerous small lateral teeth; chitinous arch broad, flat, and indistinct in middle. Spermathecae ovoid, weakly annulate or wrinkled, the individual ducts much longer than common duct.....*L. aquilonia*
- Cibarium with 4 or more acute teeth. Spermathecae otherwise 6
6. Cibarium with a well-marked chitinous arch and 4 horizontal teeth. Spermathecae with hemispherical head and finely annulate body, distinctly wider than ducts. Wings broad. Ninth tergite with anterior dorso-lateral rugose areas.....*L. cruciata* (= *diabolica*)
- Cibarium without well-marked chitinous arch. No rugose areas on ninth tergite. Wings very narrow..... 7
7. Spermathecae with hemispherical head and annulate body, with large terminal knob and slender ducts; common duct short. Ascoids short, about half length of their respective segments..
.....*L. stewarti*
- Spermathecae essentially tubular, the head oblate, the body not wider than ducts, and the terminal knob small.. 8
8. Cibarium with 2 or more small teeth between the 4 larger teeth. Spermathecal ducts slender, of even width throughout, over 2.5 times as long as stem of genital fork.....
.....*L. vexatrix vexatrix*
- Cibarium without small intercalated teeth between the 4 major teeth.... 9
9. Spermathecal ducts more slender and faintly annulate on distal fourth, over 7 times length of stem of genital fork. Ascoids short, not reaching ends of segments.....*L. oppidana*
- Spermathecal ducts less markedly narrowed distally, more faintly annulate, not over twice length of stem of genital fork. Ascoids long, extending well beyond ends of segments..
.....*L. vexatrix occidentis*

OBSERVATIONS

Following the initial finding of a few specimens of *L. shannoni* in hollow trees in swampy woodland near Pearl River, La., on June 22, 1967, adults of this species were collected in eight widely separated localities scattered throughout the State north of latitude 30° N. (Table 1). A total of 763 specimens were found, 530 males and 233 females. While 78 percent of these were collected during 16 visits to the Pearl River area (June to November, 1967; September to November, 1968), 5 to 63 specimens were found during single visits in each of the other seven areas (July, September, and October, 1967). Only two specimens (females) of *L. vexatrix* were encountered, on September 3, 1967, at Chemin-a-Haut State Park near the northern border of the State, above latitude 32° N. This locality is about 60 miles NE of Ansley, where the species was reported by Hall (1936) and where it was sought unsuccessfully on September 2 in about 50 tree hollows which yielded 5 specimens of *L. shannoni*.

TABLE 1.—Collection records of *Lutzomyia shannoni* in Louisiana, 1967–1968.

Area	Parish	Locality	Males/Females ^a
Northwest	Webster	Caney Lake, Kisatchie National Forest	4/4
North central	Jackson	Ansley, roadside resting area	4/1
Northeast	Morehouse	Chemin-à-Haut State Park	17/14
Central	Rapides	Valentine Lake, Kisatchie National Forest	4/4
East central	W. Feliciana	Audubon Memorial Park	46/17
Southwest	Calcasieu	Sam Houston State Park	3/5
South central	St. Martin	Longfellow-Evangeline State Park	35/12
Southeast	St. Tammany	Honey Island Woods, Pearl River	417/176 ^a

^a Number collected during a single visit to each locality in July, September, or October, 1967, except that for Pearl River, which is the cumulative total of 16 collections (average, 37.1 per visit), June through November, 1967, and September through November, 1968.

The insects were sought principally in forested areas, in readily accessible State Parks and National Forests, because of their known preference for sylvan areas in Central and South America. cursory search for tree holes and phlebotomines was unproductive in coastal salt-marsh areas of southern Louisiana (10 sites explored in the Barataria area in the east and the Rockefeller Foundation Refuge in the west).

All specimens collected were found by searching in natural cavities in tree trunks, i.e., holes, crevices, and hollows affording sheltered resting places. Only rarely did these include spaces between buttresses, since these are relatively poorly developed in the trees of the region compared to those found in tropical forests, where buttresses serve as favored resting places for many species of phlebotomines. Suitable cavities were found almost always in hardwood trees, so that the insects were encountered only in areas where hardwoods were present and not within pure stands of pine. Although the wide range of *L. shannoni* in Louisiana includes the western area where pines predominate, as well as the oak-gum regions flanking the Mississippi River, it was found only where hardwoods provided cavities of the type which this species apparently prefers as resting-places.

L. shannoni was found most frequently in deep, narrow clefts (Fig. 1) or in dark, hollow interiors (Fig. 2) of living trees.

Only five specimens were encountered between shallow buttresses, like the only two specimens of *L. vexatrix* that were collected. Mosquitoes were commonly seen in hollow trees that harbored *L. shannoni*. The sand flies usually occurred singly or in small numbers in the cavities, and only occasionally in abundance (27 to 73 in one tree visited repeatedly). While they were sometimes found readily, thorough



FIG. 1.—Deep crevice at base of tree, showing use of insect-repellent spray to disturb and detect concealed adult sandflies.

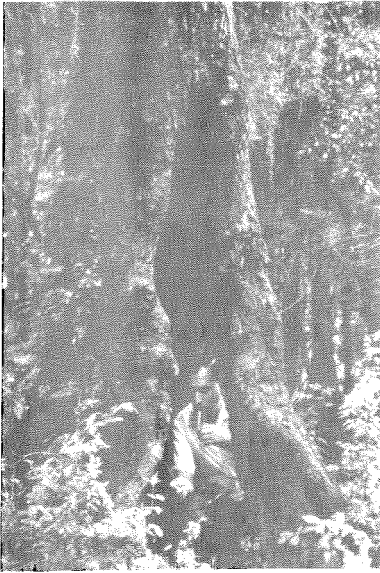


FIG. 2.—Collecting sand flies, *Lutzomyia shannoni*, with an aspirator tube inside a hollow tree (near Pearl River, La.).

searching and the use of smoke or repellent spray was usually required to detect their presence.

In areas where they were known to be present, neither the light of a Shannon trap nor the person of the collector attracted sand flies at night. A female of *L. shannoni* was observed biting the collector on only one occasion, and that was one morning in the tent in which he had slept. A rooster caged overnight in the hollow tree shown in Figure 2 seemed to increase the yield of *L. shannoni*: 113 specimens were collected from its walls the next morning, in contrast to the range of 1 to 73 found there on other occasions when unbaited. However, the 113 included only 26 females, of which 4 contained blood of undetermined origin. In Costa Rica, sand flies, including *L. shannoni*, can be collected in abundance at night by using a horse as bait.

Systematic and comparable collections were made by the same collector (R.R.) in the aforementioned hollow tree during

the third or fourth week of each month from June, 1967, through January, 1968. This series of 30-minute collections yielded successively 35, 27, 41, and 55 specimens from June through September, 10 in October, 3 in November, and none in December and late January. None was found in March, 1969, but the time of their reappearance before June was not determined.

Larvae were sought in samples of soil and debris collected in and around hollow trees which harbored adults of *L. shannoni*, by immersing and agitating the material in a saturated sugar solution, screening the flotation, and examining this and larger fragments of decayed wood with a stereomicroscope. About 30 samples processed in this way in October revealed no immature stages of phlebotomines among the dipterous and coleopterous larvae and other arthropods present. Nor did earthenware pots containing rearing medium (see below), placed in and near the adult resting places, yield eggs or larvae three weeks later.

Four attempts were made to rear *L. shannoni* in the laboratory from eggs laid by wild-caught females in September and October. Forty-two females were caged individually in moist plaster-lined vials (Hertig and Johnson, 1961). Thirty-four died within 4 days without ovipositing, and 8 produced a total of 204 eggs, ranging from 1 to 62 (average, 28) per female, laid 1 to 8 days after capture. The eggs adhered to the surface of the plaster and could be transferred to the rearing pots without risk of injury only by excising pieces of the plaster. In the earthenware rearing pots, which stood on wet absorbent cotton, more than 50 percent of the eggs hatched, all between 15 and 20 days after oviposition at a room temperature of about 26° C. Small amounts of culture medium consisting of a finely screened mixture of dried rabbit feces and decayed leaves were placed in the pots shortly before or at the time of hatching (to minimize the growth of molds), but larvae placed on this material consistently crawled

away from it onto the walls of the pot. Although some larvae appeared to contain dark ingested material, none survived longer than 72 hours or developed beyond the first instar.

Dissection of 29 females of *L. shannoni* from the Pearl River area revealed no parasites of any kind.

DISCUSSION

The search for phlebotomine sand flies in Louisiana has confirmed the previously reported presence of *L. vexatrix* (*P. vexator*) in the northern part of the State and established the widespread occurrence of *L. shannoni* in suitably wooded situations. These were the only species found by the methods used, viz., direct inspection of cavities in tree trunks during hours of daylight. A few attempts to attract sand flies to light (Shannon trap) or the person of the collector at night were unproductive, even in areas where they were known to be present, but this may have been due to unpropitious climatic or other factors at the time. Evans (1965) found no phlebotomines among 145 specimens of Psychodidae collected in a mosquito light-trap or in armadillo and rat burrows in New Orleans during the years 1959-1962, but the two specimens of *L. vexatrix* recorded by Hall (1936) were found in a mosquito light-trap in northern Louisiana.

L. vexatrix is known to range from Maryland to California across the southern States; it occurs also in northern Mexico, and, as subspecies *occidentis*, in Washington and in southwestern Canada to about latitude 50° N. (Stone *et al.*, 1965; Vargas and Najera, 1953; Harwood, 1965). Thus far only a few specimens have been collected in Louisiana, all north of latitude 32° N. Since the two herein reported were found between buttresses and not in hollow trees, and the species is known to rest in rodent burrows in other regions, the collecting methods used probably did not reveal its preferred habitat or true abundance and distribution in the State. Its natural hosts are reptiles and amphi-

bians and do not include man (Fairchild and Hertig, 1957; Chaniotis and Anderson, 1964; Harwood, 1965). It has been found to be an experimental vector of a trypanosome of toads and a potential vector of lizard malaria in California (Anderson and Ayala, 1968; Ayala and Lee, 1970).

The presence of *L. shannoni* in Louisiana is here apparently noted for the first time, although it was found in widely scattered localities in moderate numbers. The species has been previously reported (in part as *P. limai* Fonseca by Rozeboom 1944) from North Carolina and Florida to Mississippi, from Mexico through Central America, and as far south as Argentina (Fairchild and Hertig, 1950; Stone *et al.*, 1965), so that its known range extends approximately from latitude 35° N. to 30° S. While *shannoni* was not among six species of *Phlebotomus* reported from West Texas by Easton *et al.* (1968), its occurrence in western Louisiana suggests that it would also be found in the ecologically similar, forested areas of the eastern half of Texas. In Mexico it has not been reported north of the Balsas River Basin, south of Mexico City (Vargas and Najera, 1953), but the apparent gap in distribution between that region and the United States may reflect lack of exploration rather than absence of the species in northeastern Mexico.

The characteristic resting places of *L. shannoni* in the United States are hollow trees and tree-holes, while in tropical forests the adults are found more commonly between buttresses than in the less-frequent hollow trees favored by some other species (Fairchild and Hertig, 1950, who also noted that it had never been found in animal burrows). No larvae were found in October in or near hollow trees that harbored adults through the summer and fall in Louisiana, but neither have breeding places been discovered in the tropics. This species was not represented among 2,258 sand-fly larvae and pupae recovered from soil or other sites in Panama by Hanson (1961).

Only one female of *L. shannoni* was observed biting man during the course of the survey here reported, but in all probability this species, unidentified at the time, was responsible for the nocturnal annoyance by phlebotomines in the Okefenokee Swamp in Georgia reported by Johannsen (1943). It has been reported as a man-biter elsewhere and is frequently collected on horses and man in Central America. Among a variety of potential natural hosts exposed in Panama, only opossums and a chicken seemed to be attractive to *L. shannoni* (Thatcher and Hertig, 1966). A chicken placed in a hollow tree overnight seemed to augment the number of sand flies found the next morning, but we have no observations to report in regard to natural hosts in Louisiana. Opossums, as well as rodents and birds, could be expected to be available in the sites where the flies were found. While overnight campers may be subject to attack near the resting places of *L. shannoni* in the United States, the insects would generally be unrecognized as distinct from mosquitoes.

The predominance of males over females (2.4-2.9 to 1) in collections of *L. shannoni* from areas in Louisiana where more than 40 specimens were taken, and also in the 113 specimens captured in the hollow tree baited with a chicken (4.3 males to 1 female), may reflect differences in behavior of the sexes and not necessarily their relative abundance or survival. Thatcher and Hertig (1966) found male as well as female sand flies of mixed species around bait animals in a Panamanian forest: the male/female ratio in the total collected was about 0.4 (264/605), but it was 2.4 on one night, 1.2 on two nights, and 0-0.4 on 11 nights (February to May).

Although our attempts to rear *L. shannoni* from eggs laid by wild-caught females ended with the death of the first-instar larvae, the species has been successfully reared through one generation by similar methods in Panama (Johnson and Hertig, 1961). Our failure must be attributed to

some unsuitable but unidentified condition in the rearing pots.

No parasites were detected by dissection of 29 females of *L. shannoni* from one resting-place in Louisiana, but among 18 specimens collected in Costa Rica (1968) a leishmanoid flagellate was found in the stomach of one and nematode larvae in the hemocoel of two. While this latter observation may indicate a potential of the species to serve as a vector of animal—and perhaps human—parasites, it remains to be determined whether (like *L. vexatrix* in relation to a toad trypanosome and lizard malaria in California) it acts as a biological transmitter of any microorganisms in the United States.

SUMMARY

Of nine species of *Lutzomyia* (= *Phlebotomus*, in part) reported in the U.S., for which keys to adults are included, two have been found in Louisiana by deliberate search of cavities in tree trunks and collecting by means of an aspirator. Only two females of *L. vexatrix* (*P. vexator*) were found, between buttresses in northern Louisiana. *L. shannoni* was discovered in eight forested localities scattered throughout the State resting in tree-holes and hollow trees (530 males and 233 females collected, June to November). The latter species sometimes bites man, but its natural hosts are unknown. No developmental stages were recovered in October from debris, soil, or rearing pots in or near a hollow tree that always harbored adults from June to November, and breeding places remain undetermined. Eggs laid by captured females (average, 28 per female) hatched in 15-20 days, but larvae died in the first instar in standard rearing medium. No parasites were present in 29 females from one site, although flagellates or nematodes were found in 3 of 18 *L. shannoni* collected in Costa Rica.

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