THE ECOLOGY OF CUTANEOUS LEISHMANIASIS IN THE REPUBLIC OF PANAMA

Howard A. Christensen, Graham Bell Fairchild, Aristides Herrer, Carl M. Johnson, David G. Young, and Ana Maria de Vásquez

Abstract. Ecological studies on leishmaniasis at Gorgas Memorial Laboratory, Panama, over the past 4 decades are reviewed and supplemented with unpublished data relating to sand fly vectors, sylvatic reservoir hosts, parasites, and the clinical disease. The systematics, microhabitats, breeding sites, seasonality, flight activity, rearing, predators, host-feeding profiles, and vector competence of the Panamanian sand fly fauna are discussed. The reservoir hosts of the 3 indigenous species of Leishmania, *Le. braziliensis panamensis*, *Le. braziliensis amazonensis*, and *Le. hertigi hertigi*, are detailed, as well as the disease manifestations within these hosts and experimental infections. Characterization of the indigenous *Leishmania* subspecies by isoenzyme electrophoretic patterns is described and compared with that of related taxa within their respective complexes. The prevalence, clinical findings, and characterization of various forms of human cutaneous leishmaniasis in Panama, as well as diagnostic methods and treatment regimens, are outlined.

Preliminary studies of Panamanian phlebotomine sand flies (Diptera: Psychodidae) were initiated at Gorgas Memorial Laboratory (GML) in 1942, when 4 species were known from the Republic. It was not until 1949, however, that serious efforts were made to investigate the systematics, ecology, and vector potential of these insects. Since then, more than 300,000 sand flies, of 74 indigenous species from ca. 14,000 collections throughout the 9 provinces of Panama, have been identified; 5 of these species have been implicated as vectors of *Leishmania*.

Preliminary findings show that *Leishmania braziliensis* s.lat. from a sylvatic reservoir, *Proechimys semispinosus* (Spiny Rat), in the New World was accomplished at GML in 1956 (Hertig et al. 1957). Subsequently, isolations of *Leishmania braziliensis panamensis* Lainson & Shaw, 1972, believed to be the same species on the basis of hamster pathogenesis, have been made from 8 additional wild-animal taxa, including the principal reservoir host, *Choloepus hoffmanni* (Two-toed Sloth), and domestic dogs in Panama (Herrera et al. 1978a, Herrera & Christensen 1976).

Parasites responsible for human cutaneous leishmaniasis infections in the New World were reported first from Brazil in 1909 (Carini & Paranhos 1909, Lindenberg 1909) and a year later from Panama (Darling 1910). Further reports of the disease in Panama were sporadic, and studies to explore the nature and endemicity of the disease in man were not undertaken until 1956, when increased recognition of human infections helped to stimulate epidemiological investigations.

The present paper updates and summarizes data accumulated at GML over the past 4 decades on the phlebotomine fauna and on cutaneous leishmaniasis among animals and humans in the Republic of Panama.

CLIMATE AND PHYSIOGRAPHY

The Isthmus of Panama is a broadly S-shaped strip of land about 600 km long and for the most part less than 120 km wide (Fig. 1). No locality in the country is more than 65 km by air from the sea. The long axis of the isthmus lies approximately east and west so that differences in latitude from the Colombian to the Costa Rican border are too slight to affect the climate. A range of mountains, reaching elevations of over 3000 m in the west, traverses the length of the isthmus. With the ex-
cept of a few small gaps, including that through which the Panama Canal has been cut, this range is seldom less than 300 m in elevation, and it plays a dominant role in controlling the distribution of rainfall and hence the types of vegetation associations.

Panama has 2 seasons, dry and wet; the former, from January through April, corresponds to the seasonal southward movement of the NE trade winds. On the northern, or Caribbean, coast of the isthmus, the winds blow steadily from the sea, producing rain on the northern slopes of the main range of hills. Since these hills lie fairly close to the northern coast, rain falls at frequent intervals throughout the dry season. The southern, or Pacific, side of the divide receives the trade winds after they have released their rain and consequently has a more intense dry season. In the region of the Panama Canal, conditions are somewhat intermediate. During the rainy season, from May through December, winds are variable and light, and heavy thundershowers are frequent throughout the isthmus. As a result of these conditions, the annual precipitation in different areas varies widely, from about 127 cm (50 in.) to as much as 508 cm (200 in.) depending on altitude and relation to the NE trade winds. In general, the Caribbean Coast and the northern and eastern slopes of hills receive the most rain and have the shortest dry season, while the Pacific Coast, the southern slopes of hills, and areas south and west of adjacent high hills receive the least rain and have the longest dry season.

Vegetation reflects the amount and seasonal distribution of rainfall. The Caribbean Coast and the north- and east-facing slopes of hills have heavy evergreen forests. Several types of forests, depending on the amount of rainfall, elevation, soil conditions, etc., are definable, such as the tropical rain forest with well-spaced, large trees and a high, dense canopy, or the rather low, unstoried but dense scrub forest on windswept hilltops. The Pacific side of the isthmus is characterized by greater variations in vegetational cover, ranging from xero-
phytic scrub through open grassland to heavy forest with large trees and high canopy (Fig. 2–4). The conditions within the denser Pacific-side forest do not differ much from those in the rain forests of the Caribbean Coast during the rainy season. During the dry season, however, many of the trees in the Pacific Coast forest lose their leaves, allowing considerable sunlight to reach and desiccate the forest floor, so that even the heaviest of these forest types become much drier. Agricultural activities have been much more widespread and intense on the Pacific Coast, so that there are large areas where original vegetation cover has been replaced by dense second-growth scrub or, after repeated burning, by dense stands of guinea grass (*Paspalum notatum* Jac.) or even areas resembling short-grass prairie.

**PHLEBOTOMINE SAND FLIES**

**Systematics.** Panama’s singular geographical location, linking the North and South American continents, contributes to its rich and diverse phlebotomine fauna. The Republic’s proximity to the equator (10°N lat.) accounts for the dominance of Neotropical species, with fewer representatives from the Nearctic Region: only 4 species, *Luizomyia bittencourt* (Fchld. & Hertig, 1961), *Lu. tricolor* (Fchld. & Hertig, 1956), *Lu. aubertii* (Fchld. & Hertig, 1961), and *Lu. sociata* (Fchld. & Hertig, 1961), are endemic to Panama. Most of the country’s phlebotomine fauna is shared by its southern neighbor, Colombia (Young 1979). Prior to 1942, only 4 sandfly taxa had been described from Panama. Largely owing to the contributions of Fairchild & Hertig, the extent of species diversity was delineated more fully in a series of 14 publications (Fairchild & Hertig 1947 to 1961) and the number of taxa expanded to the present 74 species known from the Republic (Table 1). We have adopted the classificatory system proposed by Lewis et al. (1977) as it applies to New World taxa, which is essentially a modification of Theodor’s classification (Theodor 1965). In Panama, 3 American genera are recognized: *Luizomyia* França (66 spp.), *Brumptomyia* França & Parrot (5 spp.), and *Warbleya* Hertig (3 spp.) (Table 1). The genus *Hertigia* Fairchild is considered to be a junior synonym of *Warbleya*. Four of the most common species, *Lu. panamensis* (Shannon, 1926), *Lu. tricolor* (Fchld. & Hertig, 1952), *Lu. syphilis* (Fchld. & Hertig, 1952), and *Lu. gomezii* (Nitz., 1931), have been implicated as vectors of *Leishmania braziliensis panamensis* (Christensen & Herrer 1973), and a single species, *Lu. olmeca biolor* Fchld. &
<table>
<thead>
<tr>
<th>Genus Luizioxena</th>
<th>NO. COLLECTED (%)</th>
<th>Genus Praeintomina</th>
<th>NO. COLLECTED (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>abronis &amp; shoaele*</td>
<td>6,050 (2.1)</td>
<td>parvulus***</td>
<td>73,761 (23.6)</td>
</tr>
<tr>
<td>acridafera</td>
<td>4,425 (1.4)</td>
<td>pahos</td>
<td>453 (0.1)</td>
</tr>
<tr>
<td>australis++</td>
<td>3 (&lt;0.1)</td>
<td>pacifigemina</td>
<td>298 (&lt;0.1)</td>
</tr>
<tr>
<td>atragali</td>
<td>40 (&lt;0.1)</td>
<td>pacifemina*</td>
<td>127 (&lt;0.1)</td>
</tr>
<tr>
<td>atracochila</td>
<td>258 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>1 (&lt;0.1)</td>
</tr>
<tr>
<td>australis (-braulioharaa)**</td>
<td>16 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>1,015 (0.3)</td>
</tr>
<tr>
<td>bidentata</td>
<td>144 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>16 (&lt;0.1)</td>
</tr>
<tr>
<td>biopodetum++</td>
<td>456 (&lt;0.2)</td>
<td>pacifemina++</td>
<td>105 (&lt;0.1)</td>
</tr>
<tr>
<td>biotites</td>
<td>15 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>7 (&lt;0.1)</td>
</tr>
<tr>
<td>concorpa</td>
<td>1,352 (0.7)</td>
<td>pacifemina++</td>
<td>300 (&lt;0.1)</td>
</tr>
<tr>
<td>cupietaia</td>
<td>9,611 (2.2)</td>
<td>pacifemina++</td>
<td>29,436 (9.4)</td>
</tr>
<tr>
<td>eurycentra (-pahos)**</td>
<td>4,407 (1.6)</td>
<td>pacifemina++</td>
<td>238 (0.1)</td>
</tr>
<tr>
<td>eurycentra++</td>
<td>394 (0.3)</td>
<td>pacifemina++</td>
<td>209 (0.1)</td>
</tr>
<tr>
<td>thosigurata++</td>
<td>10 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>4 (&lt;0.1)</td>
</tr>
<tr>
<td>triacanthos</td>
<td>112 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>139 (&lt;0.1)</td>
</tr>
<tr>
<td>triacanthos++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>58,627 (18.1)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>21,246 (6.8)</td>
</tr>
<tr>
<td>tresionella</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>12,225 (3.9)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>159 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>62 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>72 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>507 (&lt;0.2)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>229 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>18 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>38,487 (12.3)</td>
</tr>
<tr>
<td>tresionella</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>10 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>10 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>10 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>10 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>10 (&lt;0.1)</td>
</tr>
<tr>
<td>tresionella++</td>
<td>128 (&lt;0.1)</td>
<td>pacifemina++</td>
<td>10 (&lt;0.1)</td>
</tr>
</tbody>
</table>

** Author given by Christensen (1972b), Christensen & Rutledge (1973), Young 1979.
*** Synonymies after Christensen & Rutledge (1973) and Young (1979).
++ Recorded in Panama since check-list of Christensen (1972b).
+++ Man-hitters.
++++ Man-hitters incriminated as vectors of Leishmania braziliensis panamensis.

Theodor, 1971, as the vector of Le. mexicana aristafer Lainson & Shaw, 1979, in this country (Christensen et al. 1972).

Cellulose-acetate enzyme electrophoresis has been used in the characterization of Lu. panamensis, Lu. yelephileter, Lu. gomezi, and another suspected vector, Lu. sanguinaria (Fchld. & Hertig, 1957). Isozyme markers of several malic acid (Me) alleles and the presence or absence of a specific hexokinase (HK) allele uniquely characterized each of these species (Petersen 1982). Biochemical methods of identifying sand flies may be used in taxonomy as an adjunct to classical methods and may play an important role when morphological characters fail, as in the case of sibling species. In Panama, distinct isozyme patterns for identical species have been observed in different geographic areas. Luizonia panamensis collected in Changuinola, Bocas del Toro Province, an area with almost no dry season, was shown by contingency chi-square tests of phosphoglucone isomerase (Pgi) allele frequencies to be significantly different ($\chi^2 = 90.5$, df = 12, $P =$...
Table 2: Panamanian phlebotomine sand flies collected in different microhabitats.

<table>
<thead>
<tr>
<th>Genus Lutzomyia</th>
<th>ArboREAL*</th>
<th>Terrestrial**</th>
<th>Crevices &amp; Caves</th>
<th>Animal Burrows</th>
<th>Human Dwellings</th>
<th>Other***</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>abdonovai</td>
<td>5,767 (8.8)</td>
<td>68 (1.0)</td>
<td>31 (0.5)</td>
<td>97 (1.8)</td>
<td>—</td>
<td>—</td>
<td>5,963</td>
</tr>
<tr>
<td>acutipennis</td>
<td>295 (0.3)</td>
<td>1 (&lt;0.1)</td>
<td>10 (0.2)</td>
<td>550 (10.3)</td>
<td>—</td>
<td>—</td>
<td>857</td>
</tr>
<tr>
<td>auberti</td>
<td>22 (&lt;0.1)</td>
<td>3 (&lt;0.1)</td>
<td>22 (0.4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>179</td>
</tr>
<tr>
<td>cayennensis</td>
<td>210 (0.3)</td>
<td>2 (&lt;0.1)</td>
<td>5 (0.1)</td>
<td>202 (5.0)</td>
<td>—</td>
<td>—</td>
<td>201</td>
</tr>
<tr>
<td>ecclesiastes</td>
<td>96 (0.1)</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>79</td>
</tr>
<tr>
<td>perniciosus</td>
<td>45 (0.1)</td>
<td>735 (12.0)</td>
<td>5 (0.1)</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>—</td>
<td>837</td>
</tr>
<tr>
<td>crassipes</td>
<td>95 (0.1)</td>
<td>—</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>85</td>
</tr>
<tr>
<td>cayennensis</td>
<td>145 (0.2)</td>
<td>38 (0.6)</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>185</td>
</tr>
<tr>
<td>keyseri</td>
<td>75 (0.1)</td>
<td>1 (&lt;0.1)</td>
<td>2 (&lt;0.1)</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>—</td>
<td>188</td>
</tr>
<tr>
<td>griseus</td>
<td>188 (0.3)</td>
<td>637 (10.0)</td>
<td>11 (0.2)</td>
<td>632 (10.5)</td>
<td>1 (0.1)</td>
<td>73 (1.3)</td>
<td>784</td>
</tr>
<tr>
<td>maccaronei</td>
<td>5,550 (8.5)</td>
<td>36 (0.5)</td>
<td>4,029 (64.6)</td>
<td>146 (2.8)</td>
<td>1 (0.3)</td>
<td>41 (0.6)</td>
<td>9,878</td>
</tr>
<tr>
<td>phlebotomus</td>
<td>1,095 (1.7)</td>
<td>114 (1.7)</td>
<td>51 (0.8)</td>
<td>7 (0.1)</td>
<td>—</td>
<td>—</td>
<td>1,267</td>
</tr>
<tr>
<td>panamensis</td>
<td>129 (0.2)</td>
<td>410 (6.7)</td>
<td>12 (0.2)</td>
<td>—</td>
<td>1 (0.2)</td>
<td>—</td>
<td>432</td>
</tr>
<tr>
<td>sanguinolentis</td>
<td>1,955 (3.0)</td>
<td>51 (0.8)</td>
<td>21 (0.3)</td>
<td>53 (0.9)</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>2,245</td>
</tr>
<tr>
<td>sorbens</td>
<td>22 (0.3)</td>
<td>159 (2.5)</td>
<td>17 (0.3)</td>
<td>13 (0.2)</td>
<td>1 (0.1)</td>
<td>—</td>
<td>121</td>
</tr>
<tr>
<td>tredecimaculatus</td>
<td>2,227 (3.4)</td>
<td>1,360 (19.9)</td>
<td>21 (0.3)</td>
<td>51 (0.8)</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>2,340</td>
</tr>
<tr>
<td>transversata</td>
<td>1,916 (3.0)</td>
<td>248 (3.8)</td>
<td>161 (2.6)</td>
<td>139 (2.2)</td>
<td>2 (&lt;0.1)</td>
<td>—</td>
<td>2,260</td>
</tr>
<tr>
<td>trinidadensis</td>
<td>177 (0.3)</td>
<td>14 (0.2)</td>
<td>21 (0.4)</td>
<td>1,401 (24.0)</td>
<td>5 (0.1)</td>
<td>—</td>
<td>1,798</td>
</tr>
<tr>
<td>vespertilibros</td>
<td>63 (0.1)</td>
<td>1 (&lt;0.1)</td>
<td>2 (&lt;0.1)</td>
<td>57 (1.0)</td>
<td>—</td>
<td>—</td>
<td>128</td>
</tr>
<tr>
<td>philiffei</td>
<td>28,528 (43.6)</td>
<td>889 (13.6)</td>
<td>1,363 (21.9)</td>
<td>55 (0.9)</td>
<td>12 (0.2)</td>
<td>6 (0.1)</td>
<td>30,845</td>
</tr>
<tr>
<td>30 other</td>
<td>—</td>
<td>—</td>
<td>21 (0.3)</td>
<td>145 (2.8)</td>
<td>—</td>
<td>3 (0.1)</td>
<td>176</td>
</tr>
<tr>
<td>Lutzomyia spp.</td>
<td>742 (1.1)</td>
<td>110 (1.7)</td>
<td>23 (0.3)</td>
<td>145 (2.8)</td>
<td>3 (0.1)</td>
<td>61 (0.8)</td>
<td>1,082</td>
</tr>
</tbody>
</table>

* Tree associates: tree trunks, tree buttresses, tree holes, hollows of trees.
** Forest floor associates: leaf litter and low-growing shrubs.
*** Old ruins, rock walls, bridges, culverts, wells, cisterns.
† Includes L. phlebotomus, since 9 are indistinguishable.
‡ Includes L. vespertilibros, since 9 are indistinguishable.

0.001) from Lu. panamensis collected at 4 sites in Panama and Colon provinces, areas of alternately wet and dry seasons. Isozyme allele frequency determinations provide a quantitative means to analyze Lutzomyia populations. Such genetic markers may be useful eventually in detecting variation in the efficacy of transmission of Leishmania by vector species.

Microhabitats: The choice of microhabitats used as daytime resting places by various species indicates rather definite preferences in certain cases, while in others little or no preference is demonstrable (Table 2). In some species that show strong preference for specific microhabitats, the presence of a particular host may be the dominant factor in their choice of resting sites. In other cases the basis for the selection may be due to a preferred breeding site, or because the microhabitat is the only suitable site in an otherwise unfavorable environment. Thus in heavy forest during the rainy season, conditions of humidity are such that suitable resting places are superabundant and any preference shown would seem to indicate a choice based on requirements other than mere shelter. In dry and open situations, on the other hand, the microhabitat chosen may be the only available shelter.
buttresses may be narrow and deep or wide and shallow, and they are occasionally as large as a small room. Often the buttresses are thin and boardlike and they may curve and twist in such a way that round or oval and vertical or horizontal holes are formed. The deeper and darker crevices seem to be preferred by sand flies, where they are associated with craneflies (Tipulidae), mosquitoes and other small Diptera, Orthoptera, spiders, harvestmen (Phalangida), pseudoscorpions (Cheloneothida), snails, land crabs, and geckoes. Tree holes and hollow trees are not as common as buttresses in the forest; however, they afford equivalent humidity and temperature conditions and accommodate a similar diversity of sand fly species. Hollow trees, when large enough to support groups of bats, are usually dominated by chiropterophilic sand fly species such as *Lu. vesperperitans* (Fechl. & Hertig, 1958) and *Lu. vesperperitans* (Fechl. & Hertig, 1947). We have collected 54 phlebotomine species from arboreal microhabitats, and it seems to be the preferred daytime resting site for about a dozen species (Table 2), including the leishmaniasis vector *Lu. ylephibina*

**Arboreal microhabitats.** These microhabitats include tree trunks, tree holes, hollows of trees, and tree buttresses. The last resting site is frequented by the greatest number of individuals and species. Buttresses are formed by the lateral extensions of the base of tree trunks (Fig. 5) and are commonly developed by many tropical trees, including species of *Ficus*, *Celtis*, and *Sterculia*. The spaces between

**Terrestrial microhabitats.** The loose accumulation of organic detritus on the forest floor is commonly several centimetres thick and provides a resting-site matrix and microclimate preferred by 6 Panamanian sand fly species (Table 2). Flies may be randomly dispersed in such litter, but they are more commonly found in small aggregations, despite the apparent uniformity of the substrate. Greatest densities usually are found along the decomposed leaves at the base of trees, especially between buttresses. The leaves of various low-growing shrubs (Fig. 6) also provide resting sites for several anthropophagic species, including *Lu. canaverali huda* Young, 1979, and the leishmaniasis vector *Lu. trapa bio* (Chaniotis et al. 1972).

**Subterranean microhabitats.** Caves appear to be generally unattractive as microhabitats and relatively few phlebotomine species, with the exception of *Lu. vesperperitans*, are encountered regularly in such resting sites (Table 2). The presence of bats in these sites, however, increases the likelihood of habitation by chiropterophilic sand fly species, as it does in the tree hollows. Some of the limestone caves in Panama (Fig. 7) are rather extensive and often accommodate hundreds of bats.

Many indigenous animals use burrows for resting or as temporary refuges. Most of the large burrows (>10 cm in diam) (Fig. 8) are those of the Nine-
banded Armadillo, *Dasypus novemcinctus*. Other mammals, such as the Conejo Pintado (*Cuniculus paca*), opossums (*Didelphis*, *Mephitis*), skunks (*Conepatus*), and various rodents, modify and use these burrows so that it is usually difficult to determine the inhabitant. Smaller burrows, dug by small rodents or lizards, rarely yield sand flies in Panama. Large burrows appear to be attractive to sand flies chiefly when occupied; the entrances of unused burrows often are veiled with spider webs and are seldom used by phlebotomines. It is remarkable that females generally constitute only a small portion of the sand flies collected from burrows; frequently these do not show evidence of recent feeding. We have taken 30 species of sand flies from animal burrows, of which several appear to show a marked preference for this microhabitat (Table 2). The predominant species collected from burrows, *Lu. carpenteri* (Fchld. & Hertig, 1953), *Lu. trimamula* (Fchld. & Hertig, 1952), *Lu. acutidens* (Fchld. & Hertig, 1952), and *Lu. campos* (Rodríguez, 1950), are nonanthropophilic.

**Artificial microhabitats.** Most Panamanian phlebotomine species appear to be exophilic and have been collected only rarely from human dwellings. An active antimalaria program is conducted by the Sociedad Nacional para la Erradicación de la Malaria in the Republic of Panama. Periodic spray applications of residual insecticides in rural homes, as well as in remote and isolated family huts, are efficiently carried out and may exert selective pressures against endophilism. Since most Neotropical sylvatic sand fly species are susceptible to desiccation, it seems highly unlikely that microclimates within human dwellings would approach their preferenda, even in small huts in the jungle environment. The rare sand fly that enters a house most likely does so in response to light and/or in search of a host rather than a resting site. Most of the specimens taken inside houses have been from biting collections or light traps.

Many man-made structures in Panama, from the most ambitious concrete fortifications to less conspicuous rock walls, bridges, culverts, wells, and cisterns, have been abandoned and long since reclaimed wholly or in part by the jungle (Fig. 9). Although most of man’s activities are incompatible with phlebotomine survival, many such abandoned works often create suitable microclimate conditions for several sand fly species, such as *Lu. cayennensis* (Flock & Abomnene, 1941), and *Lu. atroclavata* (Knab, 1913) (Table 2).

**Breeding sites.** Prior to the work by Hanson (1961) at GML, investigations of phlebotomine breeding sites in the New World had resulted in the recovery of about 60 immature specimens. The screening-flotation method for examination of soil samples used by Hanson, in addition to direct examination, yielded 2258 larvae and pupae (see
Hanson (1968). Of these, 600 subsequently were reared to the adult stage and identified. Some immature stages were collected on the surfaces of dead leaves on the forest floor, while others were found living several centimetres beneath the soil surface. About 96% of Hanson’s specimens were collected from soil samples between tree buttresses, though significant numbers were also obtained from leaf litter, animal burrows, the base of trees, and hollow trees. A single unidentified larva was collected from the refuse of an ant nest. Soil samples from tree buttresses showed the greatest diversity of taxa and included 11 species as opposed to 5 from forest floor litter, 4 from animal burrows, 2 from soil under roots, and a single species from soil at the base of trees. Adults collected from similar microhabitats indicate that there is a close correlation between daytime resting sites and oviposition sites for certain species. For other species, however, there is no apparent relationship, and it is assumed that the sites are used principally for shelter. Thatcher (1968) recovered 12 larvae in 4 arboreal sites from 6 to 13 m above the ground in Panama. These sites were characterized either as hollows or basins formed by the torking of tree branches, and all contained leaf litter. Rutledge & Mosser (1972) collected 117 larvae and pupae from soil at the base of trees in Panama and noted the ecological advantage of this breeding site because of its protection from flooding.

**Seasonality.** Seasonal changes in the tropics are far less dramatic than those in temperate regions. Monthly temperature averages differ little throughout the year and appear to play a minor role in influencing seasonal abundance of sand flies. Air temperatures at the northern entrance to the canal (Cristobal), compiled by the Panama Canal Company Meteorological and Hydrographic Branch between 1908 and 1965, showed an annual mean temperature of 26.9 °C and average annual maximum and minimum temperatures of 29.3 and 24.6 °C, respectively. Seasonal differences in overall abundance of phlebotomines are more closely associated with rainfall (Chaniotis et al. 1971b) and are best determined by light trap collections. Table 3 shows the mean number of sand flies collected per trap-night in light traps set 0 to 3 m above the ground for each month over a 30-year period. Results are based on 2,168 light trap collections, which provided 45,149 sand flies of 55 species. Collections were made at random throughout Panama. Although the sample size is adequate, the diversity of habitats and climatic conditions in various regions of the country imposes some bias. Therefore, the graph in Fig. 10 should be interpreted as demonstrating only the existence of a bimodal curve showing that the greatest sand fly abundance occurs during the early and late months of the wet season. Sand flies breed throughout the year. A few species are more abundant during the dry season but most reach their highest densities during the wet season (Table 3).

**Flight activity.** Much of the flight activity of sand flies occurs within a few metres of the forest floor, though most species have been collected higher than 18 m. Although a few uncommon species, such as *Lu. barretti* (Mang., 1942), *Lu. rayennensis*, *Lu. chiripanensis* (Dampf., 1947), and *Lu. longipalpis* (Lutz & Neiva, 1912), have never been collected in light traps higher than 6 m, most Panamanian species show vertical migration in association with trees. Certain taxa, such as *Lu. spinosa* (Flock & Abonnenc, 1942), *Lu. tropidohi*, *Lu. vlebiglitor*, and
Table 3: Seasonality of phlebotomine sand flies collected in light traps 0–3 m above ground, all provinces of Panama, 1942–1972.

<table>
<thead>
<tr>
<th>Lutzomyia spp.</th>
<th>No. (%)</th>
<th>Dry season (741 h)</th>
<th>Wet season (1427 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 &amp; 45</td>
<td>5 &amp; 5</td>
<td>3 &amp; 45</td>
</tr>
<tr>
<td>arubensis</td>
<td>2,978 (1.3)</td>
<td>770</td>
<td>1,059</td>
</tr>
<tr>
<td>lasiopsi</td>
<td>748 (1.7)</td>
<td>355</td>
<td>0.479</td>
</tr>
<tr>
<td>cornipes</td>
<td>3,085 (6.6)</td>
<td>1,562</td>
<td>2,378</td>
</tr>
<tr>
<td>verrucosa</td>
<td>895 (2.0)</td>
<td>216</td>
<td>0.251</td>
</tr>
<tr>
<td>ornata</td>
<td>2,033 (1.7)</td>
<td>11</td>
<td>0.055</td>
</tr>
<tr>
<td>alluaudii</td>
<td>1,080 (8.9)</td>
<td>1,319</td>
<td>1,780</td>
</tr>
<tr>
<td>gralis</td>
<td>6,176 (24.5)</td>
<td>470</td>
<td>0.619</td>
</tr>
<tr>
<td>matilei</td>
<td>572 (1.2)</td>
<td>186</td>
<td>0.254</td>
</tr>
<tr>
<td>flaviscutata</td>
<td>10,309 (23.5)</td>
<td>2,101</td>
<td>2,835</td>
</tr>
<tr>
<td>sanguinaria</td>
<td>415 (0.9)</td>
<td>133</td>
<td>0.260</td>
</tr>
<tr>
<td>trepalpa</td>
<td>1,253 (9.4)</td>
<td>143</td>
<td>1,942</td>
</tr>
<tr>
<td>trinidadana</td>
<td>648 (0.9)</td>
<td>117</td>
<td>0.148</td>
</tr>
<tr>
<td>terrinana</td>
<td>5,123 (4.5)</td>
<td>1,085</td>
<td>1,464</td>
</tr>
<tr>
<td>alephileus</td>
<td>1,036 (2.3)</td>
<td>175</td>
<td>0.236</td>
</tr>
<tr>
<td>40 other taxa**</td>
<td>2,959 (6.5)</td>
<td>1,209</td>
<td>1,619</td>
</tr>
<tr>
<td>Totals</td>
<td>45,149 (100.0)</td>
<td>13,954</td>
<td>18,894</td>
</tr>
</tbody>
</table>

* to = trap night (1 light trap operated for a 12-h period from 1800–0600 h).
** taxa included 36 Lutzomyia spp., 3 Brumptomyia spp., and 1 Warburga sp., none of which exceeded 0.3% of the light trap population.

Lutzomyia trinidadana (Newst., 1922), appear most often in traps set in or near the forest canopy (Table 4). Sand flies are very primitive Diptera with weak flight abilities. Chaminos et al. (1974), using the mark-recapture method in central Panama, reported that 90% of marked sand flies recaptured were within 57 m of the release point and that the maximum distance traveled was 200 m. Under natural conditions, phlebotomines characteristically progress along the forest floor or on the surface of tree trunks in a series of short flights of less than a metre and thus give the impression of hopping rather than flying. Light traps set at various distances above the ground usually are in close association with trees in the forest, since branches are used to attach the cord that suspends each trap. Because sand flies do not characteristically fly for sustained periods, we consider that most of the flies collected at significant heights above the ground reach these traps from the canopy or by ascending an adjacent tree trunk. Our observations indicate that sand flies actually ascend or descend trees in a series of short flights. This flight behavior would seem to be selectively advantageous for the sand fly, or any other weak-flying hematophagous insect, in search of a host. Most Panamanian forest mammals, excluding rodents, are arboreal or semiarboreal. Most of their foraging activities are therefore necessarily limited to the arboreal network of interdigitating branches of the canopy or understory. By using the same pathways, sand flies have a much greater opportunity of encountering a host than by random search of the forest floor.

During man-biting collections in the forest, we observed a preponderance of male sand flies resting on nearby tree trunks. This behavior may be selectively advantageous for mating as females ascend or descend the trees, and it may represent a primitive type of "mating swarm" (see also Miles et al. 1977).

Rearing. Hertig & Johnson (1961) developed colonization techniques that facilitated the successful rearing of 24 Panamanian phlebotomine species to adulthood (Johnson & Hertig 1961). Two of these, Lu. gomezi and Lu. sanguinaria, were maintained in closed colonies for 16 years (78 and 81 respective generations) before their termination in 1975. Lutzomyia longipalpis, Lu. vexilliventris, and Lu. trinidadana were reared through 21, 7, and 6 generations, respectively, using slight modification of the same technique (Christensen 1972a). The availability of parasite-free colony sand flies was essential for subsequent vector competence studies.
Table 4. Panamanian sand flies collected in light traps at different heights above the ground.

<table>
<thead>
<tr>
<th>Lutzomyia</th>
<th>NO. (%) &amp; %</th>
<th>% PER 10° AT METERS ABOVE THE GROUND* &amp; **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALL STRATA</td>
<td>0-6</td>
</tr>
<tr>
<td>abrogens</td>
<td>123 (0.2)</td>
<td>18.1</td>
</tr>
<tr>
<td>arboreata</td>
<td>2,652 (3.5)</td>
<td>45.3</td>
</tr>
<tr>
<td>barrettii</td>
<td>59 (0.1)</td>
<td>100.0</td>
</tr>
<tr>
<td>biopapua</td>
<td>177 (0.2)</td>
<td>45.3</td>
</tr>
<tr>
<td>cambodia</td>
<td>1,166 (1.6)</td>
<td>21.5</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>4,648 (6.2)</td>
<td>38.7</td>
</tr>
<tr>
<td>coreomata</td>
<td>1,551 (2.1)</td>
<td>6.5</td>
</tr>
<tr>
<td>coreomata</td>
<td>352 (0.5)</td>
<td>100.0</td>
</tr>
<tr>
<td>chlopamorus</td>
<td>66 (0.1)</td>
<td>51.0</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>367 (0.1)</td>
<td>13.3</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>5,373 (7.2)</td>
<td>42.4</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>210 (0.3)</td>
<td>8.8</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>824 (1.1)</td>
<td>26.6</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>58 (0.1)</td>
<td>100.0</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>78 (0.1)</td>
<td>71.4</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>105 (0.1)</td>
<td>62.5</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>113 (0.1)</td>
<td>64.2</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>922 (1.2)</td>
<td>43.3</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>495 (0.7)</td>
<td>42.9</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>30,425 (40.8)</td>
<td>30.3</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>1,001 (1.3)</td>
<td>37.6</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>201 (0.3)</td>
<td>9.4</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>537 (0.7)</td>
<td>14.2</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>138 (0.2)</td>
<td>59.8</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>46 (0.1)</td>
<td>13.4</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>5,729 (9.0)</td>
<td>16.6</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>586 (0.8)</td>
<td>14.9</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>9,097 (12.2)</td>
<td>61.9</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>33 (0.1)</td>
<td>15.5</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>158 (0.2)</td>
<td>16.4</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>1,409 (1.9)</td>
<td>17.0</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>343 (0.4)</td>
<td>38.2</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>209 (0.3)</td>
<td>97.6</td>
</tr>
<tr>
<td>claptostrietenii</td>
<td>74,382 (100)</td>
<td>29.8</td>
</tr>
</tbody>
</table>

* Percentages are based on comparisons of sand fly densities per trap night (m) at each stratum (trap night = 1 light trap operated for a 12-h period from 1800–0600 h).
* ** Metres above the ground (no. trap nights: 0–6 m, 2584; 6–12 m, 86; 12–18 m, 86; >18 m, 30).
** Includes Lp. shawii (not distinguishable).
† Includes Lp. spicifer (not distinguishable).
‡ Includes Lp. spicifer (not distinguishable).
§ Includes Lp. spicifer (not distinguishable).
|| Includes Lp. spicifer (not distinguishable).

involving several trypanosomatid genera (see Vector competence).

Predators. The paucity of information concerning predation on immature phlebotomine stages attests to the difficulties associated with direct observations of such stages in nature. Rutledge & Moser (1972) encountered representatives of a dozen insect orders associated with sand fly larvae in soil samples taken from the base of trees in the former Panama Canal Zone. Several of these orders contain predator species. We have observed unidentified mites, which occasionally infested GML sand fly colonies, attack and kill early larval instars. Records on the predation of adult sand flies have been very limited, and all definitive results have been derived from laboratory studies. In our laboratory the geckoes Thelodactylus recticolor and Gonatodes albogularis jussieu have fed readily on Panamanian sand fly species. A small cricket, Anaxipha gracilis (Gryllidae), was found to aggressively prey on blood-engorged sand flies in the forest leaf litter.
amensis, Lu. yelephiletor, Lu. gomezi, and Lu. trapidii, made up 26.4, 22.9, 20.1, and 17.4% of the total, respectively. Host preference studies using birds, reptiles, amphibians, and various animals from 6 mammalian families as bait in mineral oil traps showed that rodents were most attractive to Lu. panamensis, Lu. sanguinariae, and Lu. ollinco biicolor (Christensen & Herrer 1980a; Lu. vespertionis demonstrated a distinct predilection for bats. Precipitin testing of blood-engorged sand flies has shown that edentates were the predominant hosts of Lu. yeplephiletor and Lu. trapidii (Tesh et al. 1971; 1972). Recent microcapillary precipitin tests by Christensen & Vasquez (1982, unpubl. data) showed the following feeding patterns of Lu. b. panamensis vectors. Lutzomyia yelephiletor (1065 host determinations) fed primarily on sloths, 493 (46.3%); porcupines, 107 (10.0%); anteaters, 80 (7.5%); and 385 other animals in 30 families within the classes Mammalia, Aves, Reptilia, and Amphibia. Lutzomyia panamensis (129 host determinations) fed principally on rabbits, 18 (14.0%); perissodactyls (probably horses), 15 (11.6%); cows, 14 (10.9%); armadillos, 13 (10.1%); and 69 other animals in 22 families within the classes Mammalia, Aves, and Amphibia. Lutzomyia tripodi (49 host identifications) fed predominantly on sloths, 30 (61.2%); and 19 other animals in 10 families; all within the class Mammalia. Lutzomyia gomezi (33 host identifications) fed most frequently on primates, 11 (33.3%). The feeding habits of other anthropophagous potential vectors of Leishmania in Panama were as follows, Lutzomyia ovillii (Ortiz, 1952) (124 host determinations) fed principally on sloths, 40 (32.3%); anteaters, 11 (8.9%); and armadillos, 11 (8.9%). Lutzomyia yelephiletor (51 host identifications) fed predominantly on armadillos, 21

(Christensen & Herrer 1975a). This cricket also was observed actively and successfully foraging for sand flies on horses used as bait animals. Anaxipha geniculata has been collected in castor oil traps, used for sand fly collections, baited with dogs and hamsters. It appears to be a very common cricket in Panama and may contribute to the control of sand flies in nature.

Host-feeding profiles. Most of our animal-baited collections have been made from man and horses (Fig. 11, Table 5). We have recorded 30 species from man-biting collections, of which 22 species were observed to bite man in nature (Table 1). Although Lutzomyia species are primarily nocturnal, Chaniotis et al. (1971a) have reported a significant level of daytime biting by Lu. carvalhoi thula (=Lu. pessoa). Of 15,507 female phlebotomines collected biting man, the 4 vector species, Lu. pan-

![Table 5. Seasonality of Lutzomyia sand flies horse-biting activity at Quebrada Bonita, Colon Province, central Panama, 1956–1972.](image)

<table>
<thead>
<tr>
<th>Species</th>
<th>No. (%)</th>
<th>Male &amp; Female</th>
<th>Dry Season (100 tp)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Jan. through April</td>
</tr>
<tr>
<td></td>
<td>6 &amp; 8</td>
<td>8 &amp; 8</td>
<td>(%)</td>
</tr>
<tr>
<td>gomezi</td>
<td>3,221 (8.9)</td>
<td>1,275</td>
<td>32.7</td>
</tr>
<tr>
<td>panamensis</td>
<td>4,572 (12.9)</td>
<td>419</td>
<td>4.2</td>
</tr>
<tr>
<td>sanguinariae</td>
<td>12,529 (34.7)</td>
<td>4,946</td>
<td>49.5</td>
</tr>
<tr>
<td>trapidii</td>
<td>14,079 (41.7)</td>
<td>2,911</td>
<td>29.1</td>
</tr>
<tr>
<td>yeplephiletor</td>
<td>407 (1.1)</td>
<td>85</td>
<td>0.9</td>
</tr>
<tr>
<td>11 other spp.**</td>
<td>430 (1.1)</td>
<td>32</td>
<td>0.9</td>
</tr>
<tr>
<td>** Totals**</td>
<td>36,168 (100.0)</td>
<td>9,688</td>
<td>26,500</td>
</tr>
</tbody>
</table>

* tp = trap period (a 2.5-h period from 1900–2130).
** abantiae/ shamoni, biplomi, carvalhoi thula, cruxana, goniadita, labo, olmea, bozor, ovillii, serana, vesperina.
The feeding habits of *L. sanguinaria*, the 5th most common species in man-biting collections (5.8% of the total), are virtually unknown, since only 11 blood-engorged females have been taken in the field from collections other than man or horse bait. The hosts of these specimens were identified as 4 sloths, and 1 each from unidentified mammal, man, unidentified primate, identified carnivore, rabbit, porcine, and chicken. *Luizomyia showmani* (Dyar, 1929)/Lm. abaporneni (Flock & Chassignet, 1947) (females indistinguishable), the 3rd most common taxa collected in tree buttresses, are not anthropophagic in Panama, though frequent feedings on sloths [47 (28.0%) of 168] may play an important role in the maintenance of leishmanial infections among these edentates. *Luizomyia trinidadensis*, the 2nd most common species collected in tree buttresses, is primarily a reptile feeder [81 (64.3%) of 126 host identifications] and is not believed to contribute significantly to the maintenance of *Leishmania* in the forests of Panama. Armadillos were the most frequent host of *L. tri-

Armstrong [71 (74.7%) of 95 feedings], a common sand fly of animal burrows (Table 2).

**Vector competence.** One essential prerequisite for biological vectors is that they live long enough after acquisition of a parasite for the development and/or multiplication of the parasite prior to transmission. Panamanian sand flies are extremely delicate Nematocera; they are highly susceptible to desiccation and are therefore assumed to be relatively short-lived. However, adults of 3 species colonized at GML, *Lu. sanguinaria*, *Lu. gomezi*, and *Lu. vestigialis*, have lived as long as 1 month under laboratory conditions. Most other species in nature may also have a life expectancy of a month or longer, although only a small proportion of individuals probably attain this longevity owing to unfavorable environmental conditions and predators.

Workers at GML have dissected about 10,000 wild-caught sand flies in search of natural *Leishmania* infections. Flagellates were encountered in 11 of the 33 species examined, although as shown in Table 6, only 6 of 812 isolates from 4 species were proven to be *Leishmania* (Johnson et al. 1962, 1963, McConnell 1963, Schneider & Hertig 1966, Christensen et al. 1969). To date, 5 Panamanian sand fly species have been incriminated as vectors of *Leishmania* (Christensen & Herrer 1973). Of the 3 subspecies of *Leishmania* indigenous to Panama, *Lb. hertigii* Herrer, 1971, *Lb. aristedesi*, and *Lb. panamensis*, only the last has been reported from phlebotomines and man. In addition to the isolation of *Lb. panamensis* from naturally infected *Lu. gomezi*, *Lu. vestigialis*, *Lu. vestigialis*, *Lu. pan-

**Table 6.** *Luizomyia* and other flagellates isolated from wild-caught Panamanian *Luizomyia* sand flies.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. dissected</th>
<th>No. positive</th>
<th>No. of isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>gomezi</em></td>
<td>940</td>
<td>40 (4.3%)</td>
<td>1 (0.11)</td>
</tr>
<tr>
<td><em>panamensis</em></td>
<td>1,274</td>
<td>18 (1.4%)</td>
<td>1 (0.08)</td>
</tr>
<tr>
<td><em>trinidadiae</em></td>
<td>2,789</td>
<td>375 (13.5%)</td>
<td>3 (0.11)</td>
</tr>
<tr>
<td><em>vestigialis</em></td>
<td>1,128</td>
<td>101 (9.0%)</td>
<td>1 (0.09)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>6,131</td>
<td>534 (8.7%)</td>
<td>6 (0.10)</td>
</tr>
<tr>
<td>29 other species</td>
<td>5,881</td>
<td>278 (7.2%)</td>
<td>6 (0.10)</td>
</tr>
<tr>
<td>Totals</td>
<td>10,012</td>
<td>812 (8.1%)</td>
<td>12 (0.12)</td>
</tr>
</tbody>
</table>
1970) and during xenodiagnostic feeding trials on naturally infected Two-toed Sloths (Christensen & Herrer 1979). Although not considered natural vectors of members of the Le. mexicana complex, colonized Le. gomezi (Fig. 12) and Le. sanguinaria were shown to be susceptible to indigenous and nonindigenous strains (Johnson & Hertig 1970, Christensen & Herrer 1980). Leishmania b. panamensis promastigotes developed primarily in the pylorus and hindgut of these flies, as opposed to those of Le. m. aristedes, which developed predominantly in the midgut and cardia (Fig. 13).

Luizomyia olmeca bicolar is not a common species in Panama, representing about 1.7% of our total phlebotomine collections (Table 1); however, in the region of Sasardi, San Blas territory, this taxon constituted 26.8% of all sand flies collected and 99.0% of those taken in castor oil traps baited with Rice Rats, Oryzomys capito, and Spiny Rats, Proechimys semispinosus (Christensen et al. 1972). These rodents were the principal reservoirs of Le. m. aristedes in Sasardi (Herrer et al. 1971), the only focus of this parasite discovered in Panama to date, and Lu. olmeca bicolar was implicated as the vector of this zoonosis on epidemiological evidence. Nothing is known of the vector(s) of the porcupine-specific Le. b. hertigi, although Lu. yephitera and Lu. shannoni/Lu. absoncrea are known to feed on this rodent fairly frequently (10.0 and 11.3%, respectively) in Panama (Christensen & Vasquez 1982, unpubl. data).

Identification of flagellates other than Leishmania isolated from sand flies has been partially successful. Ultrastructural comparisons of promastigotes isolated from Panamanian phlebotomines by Wallace & Hertig (1968) indicated that at least one isolate was probably Crithidia.

Xenodiagnostic studies at GML, using laboratory-reared sand flies to feed on newly captured sloths, have shown that the intraerythrocytic flagellate Endotrypanum schauinslandi Mesnil & Brimont, 1908, establishes heavy infections in 3 species of Panamanian sand flies, viz., Le. gomezi, Le. trapidoi, and Le. sanguinaria (Christensen & Herrer 1976). Some trypanosomal infections of Panamanian Le. trombodiensis are due to Trypanosoma thecadactylti Christensen & Telford, 1972, acquired from forest geckoes, Thecadactylus rapicaudus (Christensen & Telford 1972). Most of the flagellate infections in Panamanian Le. vespertilionis are probably acquired from bats. Laboratory-reared Le. vespertilionis that fed on Sheath-tailed Bats, Saccopteryx bilineata, infected with T. leonisaleanei Zeledon & Rosabal, 1969, developed heavy infections (Christensen & Herrer 1975b).

RESERVOIRS

The first isolation in the New World of a parasite from a sylvatic animal that caused cutaneous leishmaniasis in man was accomplished by workers at GML in 1956 (Hertig et al. 1957): the Leishmania was isolated in culture from the blood of a Spiny Rat, Proechimys semispinosus, captured in the vicinity of the northern entrance to the Panama Canal. Parasites, believed to have been Le. b. panamensis on the basis of hamster pathogenesis, were isolated from the blood of 21 (10.5%) of 200 Proechimys collected during 1956 and 1957 (Hertig et al. 1958) in 6 endemic foci in central Panama and from a single Hoploerythrus gymnotus (Armed Rat). Remarkably, no additional isolations from these animals have resulted from the blood cultures of ca. 900 Proechimys examined since 1958.

Continued investigations in search of Panamanian reservoirs have expanded the known indigenous hosts of Leishmania to include dogs and 14 sylvatic animals (Herrer et al. 1973a). Apart from the 21 positive blood cultures from Proechimys and 1 Hoploerythrus, Le. b. panamensis has been isolated from 11 (3.3%) of 335 dogs, 1 (1.6%) of 64 Aotus trivirigatus (Night Monkey), 1 (1.2%) of 87 Seguinus geoffroyi (Geoffroy’s Tamarin), 1 (12.5%) of 8 Nasua nasua (Southern Coati), 3 (2.5%) of 119 Potos flavus (Kinkajou), 1 (11.1%) of 9 Bassariscus astutus (Bushytailed Olingo), 2 (1.2%) of 163 Bradypus minimus (Three-toed Sloth), and 110 (19.4%) of 566 Choloepus hoffmanni (Two-toed Sloth) (Fig. 14). The Two-toed Sloth is considered to be the principal reservoir of Le. b. panamensis in Panama, and the other sylvatic animals from which we have isolated the parasite to be ancillary hosts (Herrer & Christensen 1980). The parasite has been cultured from the blood, skin, and viscera of Choloepus and is most frequently isolated from the spleen (10.2%) and nose (7.9%) by culturing triturated biopsies from these sites in Senckel’s modified medium (Herrer et al. 1966). We have never observed in these animals any abnormalities of the skin or viscera, such as depigmentation, lesions, or incrustations, indicative of Leishmania; infections in both sloth species are completely cryptic (Herrer & Christensen 1975).

Dogs may serve as incidental reservoirs of the disease and/or as liasons of the infection between the jungle and rural settlements in the Republic (Herrer & Christensen 1976).
In 1968 the first isolation of *Le. m. aristedesi* in Panama was made from a Rice Rat, *Oryzomys capito* (Fig. 15), at Sasardí, San Blas territory, near the Caribbean Coast (Herrer et al. 1971). Further investigations revealed a zoonotic focus of the disease, which affected 36% of *O. capito*, 43% of *P. semispinis*, 1 of 35 *Marmosa rhabdota* (Brown Murine Opossum), and 1 of 2 *Agouti paca* (Paca) examined in this area. The disease was manifested differently in each of the host species. Infections in the principal reservoir host, *O. capito*, were mostly confined to the dorsal aspect of the tail and resulted in 1 or more depigmented areas 3 to 7 mm in diam. Parasites were isolated from a tail lesion in the *Marmosa* that resembled a typical cutaneous lesion seen in humans, *Leishmania m. aristedesi* was isolated from *Proechimys*, primarily from inapparent infections of the ear pinnae. The disease was not observed among any of the many local Cuna Indians nor in the few Chocó Indians in the region. To date, despite extensive investigations in many regions of the country, Sasardí remains the only focus of *Le. m. aristedesi* discovered in the Republic, and the parasite has never been isolated from humans in Panama.

The 3rd *Leishmania* subspecies indigenous to Panama, *Le. h. hertigi*, was isolated from the skin of a Prehensile-tailed Porcupine, *Coendou rothschildii* (Fig. 16), in 1965 and reported as a new species 6 years later (Herrer 1971). *Leishmania h. hertigi* has a remarkably high prevalence rate among Panamanian *Coendou* (ca. 89%) and appears to be host specific for porcupines, since it has never been isolated from another animal species in this country, including man. The parasites are disseminated cryptically throughout the skin and also have been isolated frequently from the liver and spleen and rarely from the heart blood of these animals.

**Leishmania Characterization**

The 3 indigenous *Leishmania* subspecies can be differentiated by their pathogenesis in experimentally inoculated Golden Hamsters, *Mesocricetus auratus*. A concentration of $10^5$ *Le. h. panamensis* promastigotes inoculated intradermally in the nose of hamsters results in a slight swelling with few parasites. The swelling attains maximum size after 4 to 6 weeks. Within 6 months the infection spreads to other areas of the body, including ears, feet, and tail, where it may manifest itself as a slight swelling and/or produce a slight redness; often areas to which the infection has spread appear normal and parasites are detected only by culture (Herrer et
Fig. 17. *Mesorictus auratus* (Golden Hamster) with enlarged nose owing to experimental intradermal inoculation of *Leishmania mexicana aristedesi* and showing metastasis to feet and tail 17 months postinoculation.

*Leishmania m. aristedesi*, in contrast, produces a large swelling, rich in parasites, in the nose of hamsters inoculated with the standard inoculum of promastigotes; the swelling attains maximum size in 2 or 3 months. Metastasis to other parts of the body occurs after 3 to 5 months and produces histiocytomas containing large numbers of amastigotes (Herrera et al. 1973b: Fig. 17). Identical concentrations of *L. h. heirtzi* promastigotes inoculated intradermally into the nose of hamsters produce transient infections of several months to 1 year without swelling or dissemination of the parasites.

In addition to characterization by hamster pathology, the 3 *Leishmania* taxa may be separated by their distinct electrophoretic mobilities for any of the following 9 enzymes by using the cellulose-acetate membrane technique: acid phosphatase (Acp), alanine amino-transferase (Ala-t), aspartate amino-transferase (Asa-t), glucose 6-phosphate dehydrogenase (G6pdh), glutamate oxaloacetate transaminase (G0t), hexokinase (Hk), 6-phosphogluconate dehydrogenase (6pgdh), phosphoglucone isomerase (Pgi), and fructokinase (Fk) (Keutner & Christensen 1980).

It is somewhat more difficult to separate the subspecies within the respective complexes proposed by Lainson & Shaw (1972). Although pathogenesis in hamsters appears to be similar for all members of the *Leishmania braziliensis* complex, we have been

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Fig. 18. Cellulose-acetate membrane electrophoresis demonstrating the different mobilities of the enzymes: a, 6-phosphogluconate dehydrogenase derived from promastigotes of
able to distinguish the indigenous *Le. b. panamensis* from the South American *Le. b. braziliensis* Vianna, 1911, and *Le. b. guyanensis* Flock, 1954, on the basis of distinct electrophoretic mobilities for the enzyme 6Pgdh (Fig. 18a). Gardener et al. (1974) reported electrophoretic mobility differences in the enzyme malic dehydrogenase (Mdh) between the LV 41 "*Le. mexicana*" strain from Panama and the South American *Le. m. amazonensis* Lainson & Shaw, 1972.

Lainson & Shaw (1979) proposed a new subspecific rank, *Le. m. aristedesi*, for the Panamanian parasite. Recently, we were able to electrophoretically distinguish the indigenous *Le. m. aristedesi* from the South American *Le. m. amazonensis*, as well as *Le. m. mexicana* Biagi, 1953, from Belize. *Leishmania*
m. aristedes) has a more rapid anodic migration for
the Alat enzyme than does Le. m. amazonensis, and
a slower anodic mobility than does Le. m. mexicana
for the enzyme Pgm (Fig. 18b). Additionally, Le.
m. aristedes shows slower anodic mobilities than do
either of the other subspecies for malic enzyme
(Fig. 18c). Also, we have observed differences be-
tween Le. m. amazonensis and Le. m. aristedes in their
ey early pathogenesis in hamsters; the former pro-
duces a much larger swelling of the nose over com-
parable periods after intradermal inoculation of the
standard concentration of promastigotes (Fig.
19–22). The promastigotes of both taxa used to
inoculate the hamsters were harvested from the
1st subculture isolates of hamster amastigotes from
previously infected animals. It is possible, howev-
er, that the previous in vivo and in vitro histories of
these strains may affect their pathogenesis. Lainson
& Shaw (1977), in their original description of Le.
h. deanei from Brazil, noted that this subspecies
differed from the Panamanian Le. h. herigi in its
electrophoretic mobility patterns for the enzymes
Pgi and G6pdh, as well as in its larger amastigotes.

**LEISHMANIASIS IN HUMANS**

Only 31 cases of cutaneous leishmaniasis were
reported in the Republic of Panama between its
initial detection there by Darling (1910) and 1952.
Twenty-five new cases were reported the following
year (Calero & Johnson 1953); several years later
about 100 cases were reported from the district of
Buenas Aires along the transisthmian highway in
central Panama (Hertig et al. 1958).

**Incidence.** The yearly incidence of human cut-
aneous leishmaniasis in Panama fluctuates consid-
erably; however, we have seen a general increase
in the number of cases in recent years. It is difficult
to determine whether this increase is the result of
more frequent transmission of the disease or grea-
ter awareness and improved recognition of leish-
maniasis by physicians and scientists. A total of 658
cases have been diagnosed at the GML clinic during
the past 4 years (8 = 165 cases per year). Al-
though leishmaniasis is contracted throughout the
year, most cases are acquired during the dry season
and early wet season from January through July.
This is a period of increased slash-and-burn agricul-
tural practices in which farmers enter forested
areas to work additional land for crops and raise
cattle, at which time they are exposed to sylvatic
foci of the disease. Outbreaks of leishmaniasis are
sporadic and occur throughout most of the for-
ested areas of the country. In many of these epide-
mics, the foci are rather limited in area and the
incidence of the disease declines severely after a
year or two. Other areas in central Panama, such as
Cerro Azul, Portobelo, Capira, Pacora, Chepo,
and Arraijan (Fig. 1), have had hyperendemic leish-
maniasis for many years, and inhabitants of these
high-risk localities constitute most cases diagnosed
at our clinic. The tropical rain forest within the
U.S. Army military reservation of Fort Sherman,
adjacent to the northern entrance to the Panama
Canal, also supports a natural sylvatic focus of leish-
maniasis. Takafuji et al. (1980) reported that 10
(1.6%) of 627 soldiers participating in jungle war-
fare training in this area during November 1977
contracted cutaneous leishmaniasis. Periodic sur-
veys in the forested regions of western Bocas del
Toro Province and eastern Darien Province also
have shown communities where leishmaniasis has
remained hyperendemic since their inception. Pa-

tients from these areas, however, are seldom seen
at the GML clinic because of the lack of transpor-
tation from such remote locations.

**Clinical findings.** The pathology of cutaneous
leishmaniasis caused by Le. b. panamensis in Pan-
amo, the only species reported to date in humans
in this country, is generally not as severe as that
produced by South American strains of Le. b. bra-
zilensis, since an average of only 5% of the cases
show mucocutaneous involvement (Fig. 23). The
disease is first manifested by the appearance of 1
or more small, erythematous nodules, usually 3 to
5 weeks following exposure to infected sand flies.
The nodules are single or multiple (ca. 50% of the
cases) and evolve over the next several weeks to
round, craterlike lesions of 1 cm or larger with

elevated borders (Fig. 24). If untreated, lesions en-
large to several centimetres (Fig. 25) and frequent-
ly cause slight enlargement of local lymph nodes
demonstrable by palpation. We have not been able
to determine if some infections are self-limiting,
since many people treat themselves with a variety
of home cures that include Mercurochrome®, cattle
dung poultices, leaves and tree bark, toothpaste,
battery acid, and cauterization with a heated spoon
or machete. We have recorded cases, however, that
have persisted for 30 years, often resulting in se-
rious complications. Some cases are more resistant
to treatment than others (recidivans), producing
adjacent satellite lesions and/or fresh lesions de-
veloping within cicatrizied areas. Such infections represent ca. 9% of the cases seen and often require several courses of treatment. A rare manifestation of Panamanian leishmaniasis is the hematogenous form of the disease (observed in only 4 patients) marked by the occurrence of ulcerative lesions over a major part of the body (Fig. 26). Multiple lesions may possibly result from multiple bites by infected sand flies. Peasants often remove their shirts in the jungle. However, some of the lesions occur also on the buttocks where clothing would preclude phlebotomine biting. One of the patients exhibited only 2 lesions (forehead and neck) on his arrival at our clinic. During the course of treatment, with meg-lumine antimoniate, ulcerated lesions erupted spontaneously over his entire body. *Leishmania b. panamensis* was isolated from several of these lesions.

**Diagnosis.** The preferred diagnostic procedures are accomplished by obtaining specimens via a punch biopsy (Fig. 27) or, more commonly, by abrading the skin after cleaning at the outside base of the rolled border of a lesion with a sterile lancet. The exuding serum is collected with a sterile spatula, mixed in 1 ml sterile saline and antibiotics (500 units penicillin and 1 mg streptomycin per 1 ml saline), and inoculated into Senejke's medium or brain-heart infusion blood agar slants. Direct smears are also made from the abraded area and stained with Giemsa for detection of amastigotes. An alternate method for isolating parasites, used
less frequently, is accomplished by inserting 20-ga
necules into the border of the lesion and collecting
the small amount of serum by aspirating with a 10-
ml syringe. Montenegro skin tests are frequently
performed on patients. A concentration of 10⁶
killed promastigotes in 0.1 ml of coca solution are
inoculated intradermally into the ventral skin of
the forearm. The test is considered positive after
48 h if an area of induration 5 mm or greater
develops. Using the methodology developed by
Walton et al. (1972), indirect fluorescent antibody
tests were performed on 140 patients positive for
leishmaniasis by culture and/or Giemsa-stained
smears, with the following results: negative (10.0%),
1:8 (8.6%), 1:16 (22.9%), 1:32 (27.1%), 1:64
(22.1%), 1:128 (7.1%), and 1:256 (2.1%). Monte-
negro skin tests performed on 100 of these patients
were positive in 93 of the cases.

Treatment. Cycloguanil pamoate (Camolar®),
in an oleaginous vehicle of 40% benzy1 benzoate
and 60% castor oil and administered as a single
350-mg intramuscular injection in adults, has been
used on a limited basis, producing complete heal-
ing in 19 of 26 cases (Johnson 1968). The single
injection of this drug has a distinct advantage over
antimonial drugs, which require multiple injec-
tions over an extended period; however, Camo-
lar® does not achieve cure rates as high as anti-
monials. The pentavalent antimonial, sodium
stibogluconate (Pentostam®), has been used effect-
ively at our clinic, but meglumine antimoniate
(Glucantime®) is our current drug of choice.

Fig. 26. A rare form of cutaneous leishmaniasis in Panama,
in which multiple-ulcerated lesions develop over much of the
body.

Fig. 27. Punch biopsy taken from the outer rolled border
of a leishmanial lesion for culturing.

provided by the Ministry of Health of Panama,
with instructions for its administration at their local
health center or hospital. The treatment regi-
men recommended by GML is 3 g Glucantime®
intramuscular daily or every 48 h for 12 doses.
Therapy for children is 80 mg/kg daily or every
48 h for 12 doses, the total dose not to exceed
3 g.

SUMMARY AND CONCLUSIONS

Important achievements by GML researchers in
ecology of leishmaniasis studies over the past 4
decades involving vectors, reservoirs, and parasites
include: (a) collection and description of 33 new
sand fly species; (b) implication of 4 Le. b. pan-
amensis vectors, Lu. panamensis, Lu. trapidis, Lu. go-
mezii, and Lu. ylephiletor, and a single potential vec-
tor of Le. m. aristides, Lu. olmea bicolor; (c) first
isolation in the New World from a sylvatic reser-
voir-host of a parasite responsible for human cut-
aneous leishmaniasis; (d) development of the

tissue-biopsy culture technique for the isolation of
Leishmania from reservoir-hosts; (e) incrimination
of the Two-toed Sloth (C. hoffmannii) as the prin-
cipal reservoir-host and 14 other indigenous mam-
als as ancillary hosts of Leishmania; (f) isolation
and description of Le. h. hertigi as a new Leishmania
species; (g) discovery of the distinctive growth pat-
terns in the intestinal tract of sand flies of members
of the mexican (midgut development) and brazili-
tensis (hindgut development) complexes (see Kil-
lack-Kendrick 1979); and (h) discovery of a zoono-
ric focus of Le. m. artraredes in eastern Panama,
thus bridging the disjunct distribution gap of
the mexicana complex between Belize and Brazil.

Adler (1964) noted prophetically that the
epidemiology of cutaneous and mucocutaneous
leishmaniasis in Central and South America would
tax the ingenuity and industry of investigators for
a considerable time to come. He noted also that the
leishmanias of mammals have speciated more in
the New World than in the Old World and that
each species may well have its own vectors, reservoirs,
and spectrum of host infectivity. The signif-
icant contributions of GML scientists to leish-
maniasis research exemplify the wisdom of Adler's
predictions.

Much progress has been made in the field of
leishmaniasis ecology in the Neotropics since the
original description of Leishmania braziliensis by
Vanina (1911). Current workers recognize as many
as a dozen species and subspecies of cutaneous
leishmaniasis in the New World and are cognizant
that additional taxa may exist (see Lainson & Shas
1979).

Recent technological advances, including
electrophoretic isozyme analysis, DNA buoyant density
analysis, and excreted factor serotyping, provide
important tools for future investigations on the
ecology of leishmaniasis.

Acknowledgments. This work is dedicated posthumously to Dr.
Marshall Hargett in recognition of his pioneering work at Gorgas
Memorial Laboratory on Leishmania, which contributed signif-
ificantly to the scientific understanding of the epidemiology of
New World leishmaniasis. We extend our thanks to Nestor Gonzalez,
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Literature Cited


Calero, C.M. & C.M. Johnson. 1953. Cataneous leishmaniasis in
the Republic of Panama: A report of twenty-five cases.

Carini, A. & U. Paranhos. 1919. Identificacion de las lepra de
Bauru en el Río de Janeiro. Rev. Med. Sao Paulo 12: 111-
16.

1971a. Daily and seasonal man-biting activity of phlebo-
1971b. Horizontal and vertical movements of phlebo-
11: 360-67.

Chaniotis, B.N., J.M. Neely, M.A. Correa, R.B. Tesh & K.M.

Johnson. 1971b. Natural population dynamics of phlebo-

1972. Diurnal resting sites of phlebononeum sandflies in a

Christensenn, H.A. 1971. Colonization of Lutzomya trinidad-
Entomol. Soc. Am. 65: 683-86.

1972. Check list of phlebononeum sand flies (Diptera: Psy-
chodidae) of Panama including two species not previously

seal animals to vectors of leishmaniasis in Panama. Am. J.


1973b. Lutzomya peruviana (Diptera: Psychodidae): poten-
tial vector of cutaneous trypanosomiasis in Panama. J. Med.
Entomol. 12: 177-78.

1976. Neotropical sand flies (Diptera: Psychodidae): inver-
sebrate hosts of Lutzomyia longipalpis (Kinetoplasida:

1977. Susceptibility of sand flies (Diptera: Psychodidae) to
Trypanosomatidae from two-toed Shoks (Edentata: Bru-

1980a. Panamanian Lutzomya (Diptera: Psychodidae) host

1980b. Development of a Panamanian strain of Leishmania
major in co-indigenous Lutzomya squamata and Lu.

mania braziliensis s. lat., isolated from Lutzomyia panaiensis

1972. Isotopic cutaneous leishmaniasis in eastern Panama
66: 53-60.

Entomol. 10: 314.

Christensen, H.A. & S.R. Telford, Jr. 1972. Trypanosoma trinidad-
dadens, sp. n., from forest geckos in Panama, and its
development in the sandfly Lutzomyia trinidadens (Neustadt)

Christensen, H.A. & A.M. de Vasquez. 1982. The tree-biess
biotope: a vertical distribution of Leishmania braziliensis


Fairchild, G.B. & M. Hertig. 1947. Notes on the Phlebo-
noneum of Panama (Diptera, Psychodidae). I. The subgenus

1948. Notes on the Phlebozone of Panama (Diptera,
Psychodidae) II. P. crassifrons Coq., (Trinidad) Newst., and

1948b. Notes on the Phlebozone of Panama (Diptera,
Psychodidae) IV. P. Ashtoniana Knab, P. raynersoni Fliesh
and Abneyne, P. chitifusius Dumpl. and some-related forms
from the West Indies and Mexico. Am. Entomol. Soc. Am.
41: 455-67.

1950. Notes on the Phlebozone of Panama (Diptera,
Psychodidae) VI. Phlebozone scopenu Dray and related species.

1951a. Notes on the Phlebozone of Panama (Diptera,
Entomol. Soc. Am. 44: 399-421.

1951b. Notes on the Phlebozone of Panama (Diptera, Psy-


Tesh, R.B., B.N. Chaniotis, B.R. Carrera & K.M. Johnson. 1972. Further studies on the natural host preferences of...


