

The Breeding Places of *Phlebotomus* in Panama (Diptera, Psychodidae)¹

WILFORD J. HANSON

Gorgas Memorial Laboratory, Panama, R. de P.

ABSTRACT

An intensive and prolonged search for the larvae of *Phlebotomus* sand flies in a variety of natural habitats in Panama resulted in the collection of 2258 larvae belonging to at least 15 of the 60-odd species now known from the area. A number of the species are quite specific in their habitat requirements, some living on the surface of fallen

dead leaves on the forest floor while others burrow an inch or more beneath the soil surface. Methods used include a sugar solution flotation technique, which is described, and simple searching of likely habitats. The scanty published information is reviewed.

Although *Phlebotomus* sand flies have received much attention because of their transmission of *Leishmania* and other disease organisms, surprisingly little is known of their breeding places. This is true even of the species of the Old World (except perhaps *P. papatasi* (Scop.) and *P. argentipes* Ann. and Brun.), where work with *Phlebotomus* has been carried on for many years.

Perhaps the first *Phlebotomus* larvae ever taken from natural habitats and recognized as such were those found by an assistant of Grassi in a cellar in Rome in 1908. Several adults were reared from these larvae and described by Grassi (1908) as a new species, *P. mascittii*.

Early investigations by Marett (1910, 1913, 1915), Newstead (1911), and Whittingham and Rook (1923) in Malta, Howlett (1913) and Mitter (1919) in India, and King (1913, 1914) in the Sudan, turned up only a few larvae and pupae from natural habitats. Many searches by these workers and others proved fruitless, and the location of breeding places was often merely assumed because of the proximity of resting adults.

It was not until the work of McCombie-Young et al. (1926) in India that substantial numbers of larvae and pupae were found. These workers were the first to apply the flotation technique in the search for *Phlebotomus*, and by this process, in which a saturated solution of sugar was used, they recovered immature stages of *P. papatasi* from soil samples taken in the vicinity of human or animal habitations.

Shortt et al. (1930, 1932) also used this method in finding the breeding places of *P. argentipes* in

Assam. They found the larvae of this species to be numerous in and around houses in the top 5 inches of soil rich in organic matter. Smith et al. (1936), while studying the breeding places of *P. argentipes*, found the larvae to be most abundant in loose soil within 20 yards of human or animal dwellings. They also used the flotation technique and recovered, besides *P. argentipes*, immature stages of *P. papatasi* (Scop.), *P. shortti* Adler and Theodor, *P. squamipleuris* Newstead, and *P. babu* Ann., which were then reared to the adult stage.

In other areas, Jerace (1939) in Italy found larvae in debris and soil cracks at or near the base of old walls. Wanson (1942) in Belgian Congo found pupae of *P. freetownensis* Sinton, *P. schwetzi* Adler, Theodor, and Parrot, *P. squamipleuris*, *P. freetownensis niger* Parrot and Schwetz, and *P. wansoni* Parrot in soil near latrines. Nájera (1946) in Spain found larvae in 14 of 130 samples of rubbish taken along streets in Madrid.

In Turkmenistan, Petrishcheva and others (1932, 1935, 1949a, 1949b, 1949c) investigated many possible habitats and obtained immature stages from the following places: land-tortoise nest, mammal burrows, dry excreta of small domesticated animals, dead leaves and rubbish, gerbil nests, and bird nests from holes in dirt banks. Soil samples from these habitats were processed by flotation in a saturated solution of NaCl. In addition, they obtained many adults by placing cages over the entrances of burrows and tree holes and over the ground.

During the course of studies of breeding places by Petrishcheva and Izyumskaya (1941) in Sebastopol, 6 tons of soil were processed, comprising 965 samples, of which 28 contained a total of 61 larvae and 91 pupae. More than 50 percent of the larvae and pupae were taken from floors of houses and animal

¹The work here reported was supported in part by a research grant from the National Institute of Allergy and Infectious Diseases, N.I.H., U.S.P.H.S. Partial cost of publication of this paper was met by Gorgas Memorial Laboratory. Accepted for publication January 12, 1961.

shelters, 32 percent from soil cracks and burrows of rodents, and 18 percent from under stones at the base of walls. None were found in dung heaps, debris under trees, litter from animal shelters, nor in rich soil from gardens.

Nothing was known of the breeding places of New World species until Ferreira et al. (1938) in Brazil reported finding four larvae in material at the base of a tree. Also in Brazil, Lutz (in Castro 1939) obtained adults in cages placed over soil of the forest floor, and Coutinho and Barretto (1941) found one larva of *P. fischeri* Pinto in soil taken at the base of a tree, and from the same material an adult of this species emerged.

Elsewhere, Pifano (1941) in Venezuela found about a dozen larvae in a hole in a wall of a house. Hertig (1942) in Peru examined material from a rock wall which was suspected to be a breeding place because of the presence of white, newly emerged adults. One pupal case was found.

In more recent years, Forattini (1954) in Brazil reared 12 adults of *P. intermedius* Lutz and Neiva from soil samples taken from a pigpen, one more of the same species from soil at the base of a bush and one of *P. pessoai* Coutinho and Barretto from soil at the edge of a stream, both in a forested area. Deane and Deane (1957), also in Brazil, obtained 32 specimens from 241 soil samples, most of which they either processed by flotation in salt solution or kept in the laboratory for emergence of adults. Three species were identified: *P. cortelezzii* Brèthes, *P. longipalpis* Lutz and Neiva, and *P. osvaldoi* Mang. They were taken from the ground in a mule shelter, under rocks, in rock crevices, from the floor of caves, and in scrapings from the trunks of two trees.

Despite the great amount of time and careful work involved, the above investigations in the New World thus turned up little more than 60 specimens, remarkably few considering the effort expended and the abundance of the adults.

The investigations reported here were begun in 1957 by the author and other members of the staff of the Gorgas Memorial Laboratory and were continued until July 1960. During this period an intensive search was carried out for the breeding places of *Phlebotomus*, with especial interest in the six common man-biting species of this area—*P. trapidoi* F. and H., *P. sanguinarius* F. and H., *P. gomezi* Nitz., *P. panamensis* Shan., *P. ylephiletor* F. and H., and *P. pessoana* Barr. Preliminary processing of soil samples by Drs. M. Hertig and P. T. Johnson of the Gorgas Memorial Laboratory, beginning in May 1957, turned up larvae of *Phlebotomus* for the first time in Panama. Since then 370 soil samples have been processed, in addition to numerous direct examinations in the field, yielding 2,258 larvae and pupae, 600 of which have been reared to the adult stage and determined. Samples were taken each month of the year, but mostly during the rainy and early dry season. Several localities on both sides of the Isthmus in or near the Canal Zone, from sea

level to about 2000 ft. elevation, were repeatedly visited for obtaining soil samples and searching for immature stages. The places most intensively investigated were: Cerro Galera, on the Pacific side of the Canal Zone; Piña Area, on the Atlantic side near the mouth of the Chagres River; Madden Forest Preserve, nearly midway across the Isthmus in the Canal Zone; and Cerro Campana, in the Republic of Panama about 50 kilometers west of the Canal Zone. Several other areas in the Canal Zone were visited, particularly along abandoned army roads where the forest is relatively undisturbed.

METHODS

The scantiness of information on breeding places of *Phlebotomus* is, no doubt, largely due to the difficulty of isolating the immature stages from the soil. Four methods have previously been used by other workers in finding breeding places: (1) careful direct examination of the soil, debris, etc., (2) placing emergence cages over suspected plots of ground, (3) keeping soil samples in the laboratory within containers, making daily observations for emerging adults, and (4) processing soil by flotation or screening or both. All of these methods require considerable time and patience, but it was found that a combination of flotation using a saturated sugar solution and washing through screens is very satisfactory for ordinary soil samples. It has advantages over methods which involve awaiting the emergence of adults either in the field or laboratory, in that all the larvae and pupae in a given sample are recovered at once. Furthermore, the process itself does not harm the larvae or pupae, which can then be reared to the adult stage and identified. Direct examination is satisfactory for dead leaves and other large objects, but it is very tedious and time-consuming if the sample consists of soil or debris.

The screening-flotation method used in this study is designed to isolate the larvae with as little extraneous material as possible. The samples being processed consist of material which falls into three categories: heavier soil particles which sink and offer no problem; live insects and other members of the soil fauna which sink in water but float in a denser liquid such as saturated sugar solution; lighter material such as bits of leaf, wood and bark, which is present in considerable quantity and complicates the search for larvae. The treatment of the screened fractions with water alone gets rid of this water-flotable material.

The details of the method in practice are as follows: For samples consisting mostly of soil and which do not contain many leaves, twigs or other large objects, (1) the soil is placed in a pan and enough saturated sugar solution added to cover it to a depth of about 2 inches. After considerable agitation by stirring with a tube through which air is bubbled, the sample is left to stand for several minutes. (2) The sugar solution with floating material is then decanted through closely woven Nylon

cloth, from which the collected material is washed into a series of three nested brass gauze sieves, 8 inches in diameter, and with 20, 40, and 60 meshes per inch. With samples containing many leaves or other large floatable objects, the preliminary flotation is omitted and the sample is washed with running water directly into the screens. (3) Most of the larvae are retained by the 40- and 60-mesh screens. These fractions are washed separately into 500 ml. cylinders, which are then filled with water. After allowing a few minutes for any larvae to settle, the water is poured off, thus eliminating the bits of wood, leaves and other water-floatable material. (4) The cylinders are then filled with sugar solution and left for about 10 minutes to allow the living organisms to rise to the surface and the heavier particles to sink. (5) The sugar solution is decanted from the cylinder through a nylon cloth strainer, which, together with the collected sugar-floatable material, is then transferred to a petri dish for examination under the microscope. Enough clean sugar solution is added to the petri dish to allow the living organisms to float. The cloth strainer, a little larger than the petri dish, is crimped radially with four staples so that it lies saucerlike in the petri dish. This is important for rapid microscopic examination, as the gently sloping cloth prevents excessive accumulation around the edge of the surface of the liquid.

The entire washing-flotation process requires at least half an hour, or longer if much organic material is present. Sugar is used instead of salt or other chemicals because it is not toxic to *Phlebotomus* larvae. Most of the larvae are collected on the 40-mesh sieve, the first-instar larvae usually passing on to the 60-mesh sieve. No real attempt has been made to recover eggs. They readily pass through the 60-mesh sieve and only two have been recovered.

LARVAL HABITATS

In table 1 are listed the potential breeding places

investigated for immature stages and the number of larvae or pupae recovered.

Table 2 lists by species and habitats those immature forms which were successfully reared to the adult stage and identified. The larval taxonomy has not yet reached the stage which would permit direct identification of the young forms.

Buttresses.—Most of the soil samples, each consisting of about 1 pint to 1 quart of soil, were taken from sheltered areas between tree buttresses, which are outgrowths at the bases of several species of tropical trees. These buttresses may be small and inconspicuous or so large that the space between them equals that of a small room. They often form narrow, deep crevices, or provide a wide variety of other situations, in most cases giving a certain amount of protection from sunlight, rain, and wind. Associated with buttresses are communities of animals similar to those found in hollow trees and rock crevices. Certain species of snails, spiders, scorpions and other arachnoids, crickets, moths, crane flies, mosquitoes, as well as *Phlebotomus* are typical inhabitants, at least during the day. The intensive collecting of adult sand flies by Drs. Fairchild and Hertig since 1943 has shown buttresses to be the richest in species of *Phlebotomus* of all habitats investigated. At least 47 species have been taken there, but the bulk of the specimens were *P. trinidadensis* Newst. and *P. ylephiletor* F. and H., with smaller numbers of *P. shannoni* Dyar. The soil between buttresses often contains considerable organic matter, such as dead leaves, insect fragments, and lizard feces, and would seem to be an ideal habitat for the immature stages of *Phlebotomus*. Therefore, particular attention was devoted to this habitat in the search for breeding places.

A total of 2,123 larvae and pupae, representing at least 11 species, was recovered from this habitat. Most were in the top 2 inches of soil with a few as deep as 4 inches. On two occasions, soil samples

Table 1.—Immature stages of *Phlebotomus* recovered from potential breeding places.

Habitat	Screening-Flotation			Direct Examination
	Samples processed	Samples positive	Larvae or pupae recovered	Larvae or pupae recovered
Between buttresses	245	50	2,123	—
Forest floor, not sheltered (dead leaves)	55	19	39	37
Burrow	27	8	26	—
Hollow tree	12	2	2	—
Under overhanging roots	9	5	16	—
Base of tree	7	3	12	—
Ant-nest refuse	3	1	1	—
Tree hole	3	0	0	—
Bark	2	0	0	—
Under log	2	0	0	—
Under rock	3	0	0	1 ^a
Chicken-coop floor	1	0	0	—
Soil cracks	1	0	0	1 ^a
Totals	370	88	2,219	39

^a Pupal case.

Table 2.—Immature stages of *Phlebotomus* from natural breeding places, reared to the adult stage and identified; listed by species and habitat.

Species	Soil between buttresses	Soil from burrows	Soil under roots	Soil at base of tree	Dead leaves, forest floor
<i>panamensis</i>	—	—	—	—	8
<i>pessoana</i>	—	—	—	—	4
<i>trapidoi</i>	1	1	—	—	10
<i>ylephetor</i>	—	—	—	—	2
<i>camposi</i>	—	1	—	—	—
<i>dyspunctus</i>	23	—	1	—	—
<i>galindoi</i>	11	—	—	3	1
<i>hamatus</i>	427	5	5	—	—
<i>nordestinus</i>	6	—	—	—	—
<i>ovallesi</i>	24	—	—	—	—
<i>rubidulus</i>	1	—	—	—	—
<i>serranus</i>	35	1	—	—	—
<i>trinidadensis</i>	13	—	—	—	—
<i>vespertilionis</i>	1	—	—	—	—
Species "M" ^a	16	—	—	—	—
Totals	558	8	6	3	25

^a Now *Phlebotomus hansonii* Fairchild and Hertig. (See Ann. Ent. Soc. America 54(2): 244, 1961.)

from the top inch of soil yielded more than 200 larvae.

The results show little correlation between the numbers of larvae and adults of any given species found in buttresses. Only 13 *P. trinidadensis* larvae have been recovered there, whereas adults of that species sometimes occur in buttresses by the hundreds. It may be that this species burrows to greater depths than those sampled, as it shows a burrowing tendency in laboratory cultures. However, half of the larvae recovered were in the top inch of soil. The immature stages of *P. shannoni*, another common buttress inhabitant in the adult stage, have not been recovered; it shows a surface-feeding tendency in laboratory cultures. *P. hamatus* F. and H., of which only five adults were taken in years of collecting, overwhelmingly dominated the larval population of this habitat. Of the 558 buttress larvae reared and identified, 427 (76.5 percent) were *hamatus*. In certain areas during the rainy season larvae of this species were present in more than half of the samples taken. This species shows burrowing habits in laboratory culture, but it also was found chiefly in the top 2-inch layer. Of the common man-biting species, only one larva of *P. trapidoi* has been taken from this habitat. Two others, apparently either *P. trapidoi* or *P. ylephetor*, did not survive to yield adults. Both of the latter two species are surface-feeders in laboratory cultures.

Animal burrows.—The 27 samples from animal burrows were taken from 1 to 3 feet inside the entrance; 26 larvae representing at least four species were recovered. One larva of *P. trapidoi*, the only man-biting species taken, was recovered from loose soil and leaf fragments scraped from the floor of a burrow. Again, *P. hamatus* was the dominant species, and of the species of which adults regularly inhabit burrows one larva of *P. camposi* Rodr. was found. It should be noted that the immature stages of *P. triramulus* F. and H., which as adults often occur in burrows in great numbers, have never been recovered.

Hollow trees.—Detritus at the bottom of hollow trees have yielded but two larvae even though adults (*vespertilionis* group) are almost invariably resting inside. The larvae did not survive to the adult stage and have not been identified. The inner surfaces of hollow trees have also been investigated, with negative results.

Rock crevices.—While collecting adult *Phlebotomus* from spaces between large rocks, the author spotted one pupal case attached to the underside of one of the rocks, about a foot from the opening. No other pupae nor larvae have since been found in this habitat, despite much searching. As the pupal case was not much above the ground surface, probably the larva merely climbed up from the soil to pupate.

Forest floor; dead leaves.—In rearing *Phlebotomus* in the laboratory, Dr. P. T. Johnson noted that while some species burrowed in the food material, others always remained on top. She therefore thought it likely that these latter species would be found in nature on some surface, such as on decaying leaves and litter on the ground. This ultimately proved to be true for the several species concerned. Through the cooperation of Major R. A. Altman, MSC, U. S. Army, an area within Fort Kobbe Military Reservation was made available to us, where large numbers of *P. panamensis* had often appeared in a horse-baited mosquito trap during the rainy season. Several larvae of *P. panamensis* were obtained from a sample of leaves, debris and soil from a shaded area near the trap. This was followed up 2 days later by the direct examination in the field of moist decaying leaves, which yielded 20 larvae. Additional larvae were found in the same area on a number of other occasions. Later, a few larvae and pupae of *P. pessoana* (closely related to *P. panamensis*) were also found on decaying leaves in two separate areas in the Madden Forest Preserve, where adults of that species were very abundant. The larvae of both species were always on moist, decaying areas of the upper and lower surfaces of the leaves, but if on the lower surfaces, then only

when the leaves were lying loosely. Because of the very long caudal bristles, which in these species are held approximately perpendicular to the body, these larvae are unlikely to occur between tightly packed layers of leaves.

P. trapidoi and *P. yephiletor* are also surface-feeders in the laboratory, but many searches for larvae on the forest floor in areas where the adults were abundant yielded none until recently (April-July 1960), when particular attention was given to well-decayed leaves and leaf fragments actually on the soil surface. In this short period a total of 38 larvae and pupae were recovered from 16 out of 26 samples of this material in three different areas. The numbers in the positive samples ranged from one to six, most of them apparently either *P. trapidoi* or *P. yephiletor*, though not all were reared for identification. All of the positive samples were within 5 to 10 feet from the trunk of a large tree where many adults of these two species were resting. Samples taken at the base of such trees have never yielded larvae of either species.

In all, 76 larvae and pupae were recovered from dead leaves on the forest floor, 39 by the flotation method and 37 by direct examination of leaves in the field. Those which were reared to the adult stage comprised five species, *trapidoi*, *yephiletor*, *panamensis*, *peossoana*, and *galindoi*.

Soil cracks.—During the dry season, from about January to May, the top few inches of the soil become hard and dry and apparently unsuitable as a larval habitat, especially on the Pacific side of Panama. However, soil cracks are also present at that time, and the more moist subsurface soil thus has direct access to the air above, which would permit adults to emerge from moist soil as well as to oviposit there. King (1914) recovered larvae in the Sudan from soil cracks which are apparently the only breeding places in certain regions there. Attempts were made to find immature stages associated with soil cracks during the dry season of 1959 (March) at Cerro Galera, a forested area where both soil cracks and adult *Phlebotomus*, particularly *P. sanguinarius*, were abundant. The cracks here were $\frac{1}{2}$ to 1 inch wide and at least 8 inches deep. The hard clay soil had a thin covering of dry leaves, under which adults of *P. sanguinarius* and *P. gomezi* were found resting during the day. As clumps of the soil along the cracks were broken away and carefully examined, one pupal case of an unknown species was found about 2 inches down from the edge of one of the cracks, approximately where the soil was noticeably more moist. The specimen is unusual in that the caudal bristles of the larval exuvia are relatively short and slightly spatulate, a condition not present in any of the known reared species. To date, no more specimens have been taken in this habitat despite much search.

Other habitats which yielded larvae or pupae were soil under roots, soil around the bases of trees and an ant nest refuse pile near a buttressed tree.

As already noted, Deane and Deane (1957) in Brazil obtained larvae of two species from scrapings of tree trunks. Considerable time has been spent examining this possible habitat, particularly where *P. yephiletor* was observed resting in large numbers. However, no immature stages were found.

While domestic or semidomestic species occur in the Eastern Hemisphere and South America, none of the species in Panama can be so regarded. Although sand flies will often enter houses or fly under stilted shacks to bite at night, they do not remain and are seldom encountered in houses during the day, even in forested areas where they are abundant. This may be because huts in or near forests are too well ventilated, usually with many openings in the walls and under the eaves, and are thus unsuitable as daytime resting places. For this reason houses as well as animal sheds were largely unexplored as possible breeding places.

Although much is still to be learned, the general picture of sand fly breeding places in Panama has begun to take form. Four of the six man-biting species (*P. trapidoi*, *P. panamensis*, *P. yephiletor* and *P. peossoana*) were found breeding on decaying leaves on the soil surface in well-shaded areas, with no evidence of preference for any particular spot. Whenever larvae were found, many adults of the same species were also observed nearby, either under dead leaves, on tree trunks, or on the underside of green leaves of low plants. If more samples were examined, probably other less common species would also be found there. The immature stages of the other two man-biting species, *P. sanguinarius* and *P. gomezi*, have not been recovered, but it is very likely that these species also oviposit on the forest floor.

In the case of a few species, particularly *P. hamatus*, *P. ovallesi* Ortiz, and *P. serranus* D. and A., a preference is shown for the sheltered areas between buttresses and at times in large burrows, while the adults of these species seldom use these habitats as daytime resting places. These species, at least, thus apparently seek out such sheltered, dark places in which to oviposit, rather than at random over the forest floor.

A few remarks should be made regarding food habits of *Phlebotomus* larvae as observed in natural habitats. The top layer of soil of the forest floor, and especially between buttresses, contains feces of various animals, such as lizards, which might well be an important supply of food for *Phlebotomus* larvae, although none were actually seen feeding on such material. Fragments of bodies of various arthropods are also numerous on the ground and on dead leaves, and often make up a considerable proportion of the material taken from buttresses. These fragments probably have accumulated from the predatory activities of ants, spiders, bats, etc., inhabiting these places. On one occasion larvae and pupae of *P. serranus* were found on a dead caterpillar taken from a dark, recessed area between buttresses. As all species being reared in the laboratory here readily feed on frag-

ments of dead insects, very possibly such material is quite generally utilized as food in natural habitats.

Parrot (1932) has found dead leaves to be sufficient for the development of *P. papatasi* in Algeria, and this appears to be true also for *P. panamensis* and *P. pessoana* here. As discussed above, larvae of four species have been found on dead leaves and in some cases were actually seen feeding on them or on the micro-organisms growing on them.

ACKNOWLEDGEMENTS

The author wishes to thank those of the Gorgas Memorial Laboratory who have helped in the progress of this study, particularly Drs. Marshall Hertig and G. B. Fairchild, under whose direction these investigations were carried out. Also appreciated is the unreserved assistance given by the U. S. Army Malaria Control Unit in the Canal Zone under the command of Major R. A. Altman and, later, Capt. V. Tipton.

REFERENCES CITED

- Castro, G. M. O. 1939. Hábitos de alguns flebotomos brasileiros. *Jornal de Comercio* [Rio de Janeiro] 112(81): 4.
- Coutinho, J. O., and M. P. Barretto. 1941. Dados biológicos sobre o *Phlebotomus fischeri* Pinto 1926. *Rev. Brasil. Biol.* 1(4): 423-9.
- Deane, L. M., and M. P. Deane. 1957. Observações sobre abrigos e criadouros de flebotomos no noroeste do Estado do Ceará. *Rev. Brasil. Malariol. Doenças Trop.* 9(2): 225-46.
- Ferreira, L. C., L. Deane, and F. O. Mangabeira. 1938. Sobre a biologia dos flebotomos das zonas de leishmaniose visceral ora em estudo no Estado de Pará. *O Hospital* [Rio de Janeiro] 14: 3-6.
- Forattini, O. P. 1954. Algumas observações sobre a biologia de flebotomos (Diptera, Phlebotomidae) em região da bacia do rio Paraná (Brasil). *Arq. Fac. Hig. Saúde Públ. Univ. São Paulo* 8: 15-136.
- Grassi, G. B. 1908. Intorno a un nuovo flebotomo. *Rend. Accad. Lincei* 17: 681-2.
- Hertig, M. 1942. *Phlebotomus* and Carrion's disease. *Suppl. Amer. Jour. Trop. Med.* 22: 1-81.
- Howlett, F. M. 1913. The breeding places of *Phlebotomus*. *Jour. Trop. Med. Hyg.* 16: 255-6.
- Jerace, F. 1939. I flebotomi degli Abruzzi. IIIa nota. (Distribuzione e biologia dei flebotomi in prov. di Teramo). *Ann. Igiene* 49(5): 309-15.
- King, H. H. 1913. Notes on the bionomics of the sandflies (*Phlebotomus*) of Tokar, Anglo-Egyptian Sudan. *Bull. Ent. Res.* 4: 83-84.
1914. Observations on the breeding places of sandflies (*Phlebotomus* spp.) in the Anglo-Egyptian Sudan. *Jour. Trop. Med. Hyg.* 17: 2-3.
- Marett, P. J. 1910. Preliminary report on the investigation into the breeding places of the sandfly in Malta. *Jour. Roy. Army Med. Corps* 15(3): 286-91.
1913. The *Phlebotomus* flies of the Maltese Islands. *Jour. Roy. Army Med. Corps.* 20: 162-71.
1915. The bionomics of the Maltese *Phlebotomi*. *British Med. Jour.* 1915: 172-3.
- McCombie-Young, T. C., A. E. Richmond, and G. R. Brendish. 1926. Sandflies and sandfly fever in the Peshawar District. *Indian Jour. Med. Res.* 13: 961-1021.
- Mitter, J. L. 1919. Preliminary report on an investigation into the breeding places of *Phlebotomus (papatasi)* and *montus* in Lahore. *Indian Jour. Med. Res.* 6: 452-61.
- Nájera, L. 1946. Observaciones sobre la ecología de los flebotomos en su fase larvaria principalmente. *Rev. Ibérica Parasitol.* 6(1-2): 139-58.
- Newstead, R. 1911-12. The papataci flies (*Phlebotomus*) of the Maltese Islands. *Bull. Ent. Res.* 2: 47-78.
- Parrot, L. 1932. Nouvelles observations sur la biologie de *Phlebotomus papatasi* (Scop.). *Arch. Inst. Pasteur d'Algérie* 10(3): 407-9.
- Petrishcheva, P. A. 1932. Contribution to the biology of *Phlebotomus* of the Karakala region of the Turkmen SSR. (In Russian.) *Murghab Parasitol. Exped.* 1930. *Acad. Sci. USSR and Health Commissar' Turkmen*, in *Trud. Sov. Izuch. proizvod. Sil. Ser. Turkmen*, part 2: 143-73, 6 figs., 24 refs. Leningrad Acad. Sci. (Abstract in *Rev. Appl. Ent. (B)* 21: 98-99, 1933.)
1935. Faune, biologie et écologie des Phlébotomes de la Turcomanie. (In Russian, with French summary.) In: *Parasites, transmissieurs, animaux venimeux. Rec. Trav. 25^e Anniv. Sci. Pavlovsky 1909-34*, pp. 202-59, 8 figs., 1 folding map, 4 diagrams, 2 graphs, 12 refs. (Abstract in *Rev. Appl. Ent. (B)* 24: 179-80, 1936.)
- Petrishcheva, P. A., et al. 1949. Nests of birds as breeding places of sandflies (*Phlebotomus*). (In Russian.) *Zool. Zhur.* 28(3): 284-6. (Abstract in *Rev. Appl. Ent. (B)* 41: 19, 1953.)
- Petrishcheva, P. A., and I. M. Grokhovskaya. 1949. The burrow of the lamelliform-toothed rat *Nesokia indica baitwardi* (Thomas) as a site of mass breeding of sandflies (*Phlebotomus*). (In Russian.) *Doklady Akad. Nauk SSSR (N.S.)* 65(4): 597-9. (Abstract in *Rev. Appl. Ent. (B)* 39: 204, 1951.)
- Petrishcheva, P. A., and V. V. Gubar. 1949. The breeding of *Phlebotomus* in the colonies of the large Gerbil (*Rhombomys opimus* Licht.). (In Russian.) *Ent. Obozrenie* 30(3-4): 242-5. (Abstract in *Rev. Appl. Ent. (B)* 41: 19-20, 1953.)
- Petrishcheva, P. A., and N. G. Izyumskaya. 1941. On breeding places of *Phlebotomus* in Sebastopol. (In Russian, with English summary.) *Trav. Aca. Milit. Med. Kiroff Armée Rouge* 25: 243-54. (Abstract in *Rev. Appl. Ent. (B)* 34: 78, 1946.)
- Pifano, F. 1941. La leishmaniosis tegumentaria en el Estado Yaracuy, Venezuela. *Gaceta Med. Caracas* 48: 292-9.
- Shortt, H. E., R. O. A. Smith, and C. S. Swaminah. 1930. The breeding in nature of *Phlebotomus argentipes*. *Ann. and Brun. Bull. Ent. Res.* 21(3): 269-71.
1932. Miscellaneous experiments with *Phlebotomus argentipes* in relation to transmission of kala-azar. *Indian Med. Res. Mem. No.* 25: 90-102.
- Smith, R. A. O., S. Mukerjee, and C. Lal. 1936. Bionomics of *P. argentipes*. Part II. The breeding sites of *P. argentipes* and an attempt to control these insects by anti-larval measures. *Indian Jour. Med. Res.* 24(2): 557-62.
- Wanson, M. 1942. Sur la biologie des phlébotomes congolais. *Rec. Trav. Sci. Med. Congo Belge* 1942(1): 23-43.
- Whittingham, H. E., and A. F. Rook. 1923. Observations on the life-history and bionomics of *Phlebotomus papatasi*. *British Med. Jour.* No. 3285: 1144-51.

Reprinted from the

ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

Volume 54, Number 3, pp. 317-322 March, 1961