STUDIES IN RELATION TO MALARIA

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ISTHMIAN CANAL COMMISSION
Laboratory of the Board of Health, Department of Sanitation

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Errata in pamphlet on STUDIES IN RELATION TO MALARIA
BY S. T. DARLING, M. D.

Page 6; 24th line from bottom; Delete shown in the drawings.
Page 7; 28th line from bottom; Read coxae for cozae.
Page 8; 16th line from top; Read nape for nate.
Page 12; 8th line from top; Read anaerobic for aerobic.
Page 16; 3d line from bottom; Delete a
Page 23; 25th line from bottom; Read *malefactor*.
Page 26; 12th line from top; Read larval.
Page 26; 18th line from bottom; Read on the, for, with.
Page 27; 29th line from top; Read spermatheca for spermathecae.
Page 29; 8th line from top; Read than 38° Baume.
Page 29; 10th line from top; Read analysis for anaylses.
Page 30; 18th line from top; Read remains for remained.
Page 31; 21st line from bottom; Read passed for passes.
Page 33; 13th line from top; Read and in these are seen.
Page 33; Chart 51499; Read polymorphonuclear for ploymorphonucle
Page 34; 9th line from top; Delete as in the preceding one.
Page 35; 5th line from bottom; Read gametes in films.
Page 36; 3d line from top; Read forms.
Page 36; 21st line from bottom; Read 1 blood negative; entered hospital few days later with tertian malaria.
Page 36; 20th line from bottom; Read tertian, all ages.
Page 37; 15th line from bottom; Read tertian, all ages.
Page 37; 19th line from top; Read became for become.
Page 38; 7th line from top; Read hemopoietic.
Page 38; 13th line from bottom; Read eosinophilia.
Page 38; After last line; Mr. A. H. Jennings for the indentification of mosquitoes.

STUDIES IN RELATION TO MALARIA.

SAMUEL T. DARLING, M. D.,

Chief, Laboratory of the Board of Health.

The following observations and experiments have developed out of work directly or indirectly related to a study of some of the factors concerned in the transmission and prevention of malarial fever in the Canal Zone:

In every malarial region it is important that the varieties of mosquitoes common to that region should be recognized, their breeding habits should be studied, and a determination made of the species of anophelines hospitable to malaria, and those transmitting it. The English observers, James, Christophers, and Stephens, noticed that certain species of anophelines were natural transmitters of malarial fever, while others were rarely, if ever, found infected naturally, although it would be possible to infect them under laboratory conditions. We know that the breeding habits of anophelines vary, too, considerably, and it may be said that there is as much selection of breeding places by anophelines as there is selection of feeding grounds by fish. Trout, salmon, and bull-heads have their analogues among anopheles larvæ, some of the latter requiring fresh aerated water, or water containing much green algæ. Others are found in tree holes and the recesses of epiphytic tree plants, such as bromelias, where they prey upon other species; while others preferring fresh aerated water are so adaptable that they will flourish in sewage, streams, or in brackish water containing half its volume of sea water. Some species require an abundance of sunlight, while other sylvan species prefer shady pools in which chlorophyll-bearing algæ are relatively absent. The anophelines insusceptible to malaria may be more limited in their choice of breeding places, so that in the work of malarial-mosquito destruction the latter may be disregarded, and attention given wholly to the breeding places of those varieties responsible for the transmission of malarial fever.

With regard to man as a host, it is necessary to have some knowledge of the limits of his infectiousness, i. e., the number of sexual forms of the malarial parasite necessary to infect susceptible mosquitoes.

Besides the question of hospitable species of anophelines, there are other matters of much importance, such as latent malaria; the effect of quinine on the parasites in man; the value of various larvacides;

algacides; agents destructive to ditch grass, and a knowledge of the quality of wire screening, and the size mesh necessary in keeping out mosquitoes.

OUTLINE OF THE SUBJECTS CONSIDERED.

Anophelines of this region.
Collection of larvæ.
Breeding out mosquitoes and methods of feeding.
Biting—infecting experiments.
Estimation of gametes.
Care of mosquitoes after feeding.
Method of examining for zygotes and sporozoites.
Description of the malarial parasite in the mosquito.
Table of infecting experiments.
Notes and conclusions from table of infecting experiments.
Limit of infectiousness of man.
Notes on the bionomics of anophelines.
Effect of salt or sea water on larvæ.
Experiments with larvacides.
Experiments with agents destructive to vegetation, grass, and algæ.
Experiments with screening of various mesh.
Relative value of wire screening of various composition, based on practical tests and chemical analyses.
Note on the value of the practice of killing anophelines found in quarters and barracks.
Latent malaria.
Effect of quinine upon the parasite in mosquito and man.
The following is a list of anophelines of the Canal Zone:

Anopheles argyritarsis, R. D.
Anopheles tarsimaculata, Goeldi.
Anopheles gorgasi, D. K.
Anopheles albimanus, Wied.
Anopheles cruzii, D. K.
Anopheles apicmacula, D. K.
Anopheles punctimacula, D. K.
Anopheles malefactor, D. K.
Anopheles eiseni, Coquill.
Anopheles franciscanus, McCrack.
Anopheles pseudopunctipennis, Theob.

The above 11 species of anophelines have been collected in the Canal Zone during the past five years. They are not taken, nor do they exist in their breeding places, in anything like equal numbers. For example: Only one specimen of *A. gorgasi* has been found. Of the 11 species, the commonest ones are *A. albimanus*, *A. pseudopunctipennis*, and *A. malefactor*, but this again must be qualified by stating that the predominance of a species varies from season to season and from place to place. In certain villages, upon going through the barracks only, *A. albimanus* will be found, while in other villages from 5 to 10 per cent of the mosquitoes will be *A. pseudopunctipennis*, and at Ancon during October, 1908, 27 per cent were *A. malefactor* and 72 per cent *A. albimanus*. Mr. A. Busck, of the Bureau of Entomology, United States Department of Agriculture,

who collected and made observations on Zone mosquitoes during 1907, gave it as his opinion that *A. pseudopunctipennis* was the commonest anopheline during the period of his stay.

The necessities of the canal operations in excavating and filling change the topography of districts and localities so as sometimes to convert salt marshes into fresh-water ponds, or to make tracts of land containing few anophelines into a vast swamp in which they luxuriate. On the other hand, swamps and breeding places may be drained or filled in the work of excavation. These factors, among others, influence the number and variety of species in a locality.

The commoner anophelines of the Canal Zone may be divided into three groups:

(A) The white hind-footed group comprising: A. argyritarsis, A. albimanus, A. tarsimaculata.

(B) The leg uniformly-colored group comprising: A. pseudopunctipennis, A. franciscanus.

(C) The spotted-leg group, comprising: A. malefactor, A. apicimacula.

These groups present well marked differences in the markings of adults, in the breeding habits and anatomical characters of the larvæ, and, as will be shown, they possess varying susceptibilities to malaria.

The following are descriptions of the species of anophelines of the Canal Zone:

Anopheles argyritarsis.—Thorax with mesonotum bluish-gray, with three more or less longitudinal lines and with pale scales over the mesonotum, and sometimes traces of two dark lateral spots. The abdomen dark, dusky-brown, with a few creamy scales. Legs covered with dark scales, with some of the tarsiapically white banded; last three joints of hind legs pure white, and also apex of first; costa dark, with two distinct and several smaller pale spots.

♀ Head black, with white upright spatulate scales in front, black behind and at the sides, a tuft of white hairs projecting forward between the eyes. Eyes black; antennæ dark, with pale silky pubescence and brown hair; basal joint dark, a few patches of white scales on the first few basal joints; palpi covered with long black scales, especially along toward the base; apex pure white, and there are also two narrow white rings on the apical ends of the joints; ventrally, the penultimate joint has a number of yellowish-white scales, which sometimes seem to form almost a ring; proboscis clothed with short dark scales.

Thorax with a bluish-gray sheen, with three more or less distinct longitudinal lines, the middle one most distinct, and of a purplish hue, with pale scales scattered over the mesonotum; scutellum dark toward the middle; mesonotum deep brown; pleuræ dark, with here and there frosty tomentum (there are traces of two dark lateral spots on the mesonotum, which are clearly seen in the St. Lucia specimen).

Abdomen dusky purplish-brown, clothed with creamy yellow scales, especially in the middle region of the segments; the segments have lateral tufts of gray scales on the posterior borders, projecting from the sides; hairs long, deep bright brown; viewed with a pocket lens the abdomen is almost black in ground color; in other specimens dull yellowish reflections may be seen.

Legs yellowish, covered with dark brown scales; first two tarsi of the forelegs apically white, last two joints dark brown, four meditarsi also with small pale apical bands; mid metatarsi and first two tarsal joints with minute apical yellow bands, last two indistinctly banded; in the hind legs the last three joints are pure snow white, and also the apex of the first; ungues very dark.

Wings with the costa dark, with four distinct and several smaller white patches; there are also numerous patches of dark scales, which vary to some extent, over the wing areas; in the ♀, from which this description is taken, the fourth long vein is covered with pale dusky scales, whilst in a ♀ from St. Lucia it is creamy white; halteres with pale stem and fuscous knob. Length, 4 to 5 mm.

♂ Palpi dark brown, with scattered white scales, especially on the last swollen joints; hair-tuft pale; there is a pale ring at apex of the apical and base of the penultimate joint; antennæ brown, with brown plumes; proboscis brown and narrow. The white scales on the head extend nearly over the neck; scales on the thorax white; the larger ungues of the four feet biserrated. Length, 4 to 5 mm.

During the period in which these experiments were being conducted I received very few specimens of this species, the sources being Miraflores, Ancon, Culebra, Paraiso, and Corozal. Two specimens of *A. argyritarsis* bit a patient having one crescent to 200 leucocytes and neither became infected. The patient was possibly an unfavorable case and the experiment was not controlled by biting susceptible *A. albimanus* at the same time. On December 2 from some anophelines collected in labor cars at Corozal, one specimen of *A. argyritarsis* was found containing a malarial zygote, 29 mu. in diameter, with fine discrete pigment.

Anopheles tarsimaculata.—This mosquito resembles *A. albimanus* very closely, except for the different arrangement of the white bands on the palpi shown in the drawings. This mosquito was found to transmit malaria.

Anopheles albimanus.—This form resembles the type in all respects except that the last tarsal joint in the hind legs has a very distinct and persistent deep black basal band. The thorax is rather browner in some specimens, and there are only two white bands to the ♀ palpi. The forelegs have dark scaled femora, pale beneath, with a small white knee spot, the tibiæ dusky scaled and also the metatarsus above, pale below, apex white; the first two tarsi have yellow apical bands, the third dark, and the last clay colored; mid legs with a large white spot near the apex of the femora; mid tarsi not definitely banded, but with a faint pale band sometimes at the apex of the metatarsus; the hind legs are dark brown, with the second, third, and apex of the first tarsal joints pure white, the last joint white, with a distinct black basal band; ungues as in the type. Wings much as in the type, but the pale scales are more yellow in color. Length, ♂ 3.5 to 4.5 mm.; ♀ 4 to 4.5 mm.

This was the commonest species of anopheline received as adults or larvæ during the period embraced by this work, and was found to transmit both estivo-autumnal and tertian malaria.

Anopheles gorgasi.—Palpi as long as the proboscis, mostly black scaled, the terminal and penultimate joints light scaled except at the bases and apices; meso-thorax gray with fine brown scales, a black spot in front of the scutellum, a pair of sublateral black spots medially; wings

with the veins scaled in black and white, two very large black patches on the costa and a smaller one toward the base and a smaller one at the apex as in *A. albimanus* Weid. The rest of the wing is too much denuded to describe. Abdomen with groups of outstanding scales laterally at the apices of the segments, the dorsum closed with yellow scales on a dark brown, the lateral tufts black. Legs mostly black scaled, hind legs with the apical half of the second, the third, and the base of the fourth joints white scaled, the remainder of the fourth and basal half of the fifth segments black, the third joint with a large black patch on the underside, which reaches from near the base to beyond the middle. Length, 3.5 mm.

A single adult female of this species was collected by Mr. A. H. Jennings.

Anopheles pseudopunctipennis.—Wings much as in *A. punctipennis* Say, but the fringe with yellow spots. Legs, long, unbanded, brown, pale at the base. Fore ungues of ♂ unequal, mid and hind equal and simple.

♀ Antennæ brown, basal joint testaceous, base of the second joint pale, and also a small pale band at the base of all the following joints: proboscis dark brown, labella yellowish; palpi dark brown, densely scaled at base, apex yellow, and also two narrow yellow bands below, slightly hairy, hairs black, except at the apex, where they are yellow; clypeus dark brown. Thorax yellowish-brown (denuded), with a dark patch on each side of the mesonotum behind; metanotum deep brown; pleuræ yellowish brown, with darker brown patches. Abdomen brown, the segment paler at the base; hairy. Legs deep brown; coxæ, trochanters and base of femora pallid; knee spot pale; ungues equal and simple. Halteres with pale stem and fuscous knob.

Wings with two yellowish white spots on the upper costal border, rest of the edge black, rather densely scaled; first submarginal longer and narrower than the second posterior cell, its stem nearly as long as the cell; mid cross vein a little nearer the base of the wing than the supernumerary cross vein; posterior cross vein still nearer the base of the wing; scales of the wings disposed as follows: First long vein with three distinct large white spots, one at the base, one underneath a large costal spot, and one between; second long vein with a dark patch near its base, all the lower branch of the fork cell dark, and most of the upper; third long vein mostly yellowish-white, with two black patches, one toward the base and the other toward the tip; fourth long vein mostly pale, with two small black patches, branches of the fork cell all dark scaled; fifth long vein with a black spot near the base, rest mostly yellow, upper branch of the fork mostly dark, a small yellow spot at the apex and another toward its base, lower branch mostly yellowish, with a black apical spot; sixth vein with the basal half creamy, the apical half dark, except a small yellow patch where it joins the wing border; fringe brown, with a yellow spot at the junction of each vein. Length, 5 mm.

♂ Last two joints of the palpi swollen and clavate, pale, basal joints dark brown, densely scaled with deep brown scales, with a narrow pale band not quite as long as the thin proboscis, which is brown, with yellow labellæ; antennæ gray, with narrow brown bands and flaxen brown hairs, the apical joint about half the length of the penultimate joint; basal lobe of the genitalia simple, claspers long and thin; fore ungues unequal, the larger one uniserrated, the smaller