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This study was supported, in part, by funding under Public Law 480, Section 104(a) and was conducted at NAMRU-2.

The opinions and assertions contained herein are the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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MEDICAL ECOLOGY DEPARTMENT
PARASITOLOGY DIVISION
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ADMINISTRATIVE INFORMATION

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Commanding Officer

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DEDICATED TO

HENRY FRANK TANTALO

CHIEF HOSPITAL CORPSMAN, UNITED STATES NAVY
KILLED 20 JUNE 1964, TAICHUNG, TAIWAN

ABSTRACT

The investigations reported here are 4 fold: (1) Taiwan monkeys (*Macaca cyclopis*) were trapped and examined to determine the geographical distribution and foci of the filaria (*Macacanema formosana*). (2) The filaria-positive area was surveyed to determine the potential arthropod vectors of this filaria. (3) Suspected intermediate hosts were experimentally fed on filaria-infected monkeys and the development of the filarial larvae has been recorded for the first time. (4) Studies were made to determine microfilariae density and periodicity.

Seventy-one of the 277 monkeys trapped throughout the island (12.7 percent of the males and 27.1 percent of the females) were positive for "malaria". Moreover, 8 of 25 males (32 percent) and 17 of 30 females (51 percent) were positive for "malaria" in the filarial-positive area. The prevalence of "malaria" and microfilariae in the female monkeys were twice those in the males.

None of the filarial positive monkeys were trapped outside of the 30 square mile area in the northeastern mountains. Twenty-three of the 55 monkeys (7 of 25 males and 16 of 30 females) were positive for microfilariae in the peripheral blood. The 254 monkeys trapped outside of this area are reported negative for filariae.

In the filarial positive monkeys a marked nocturnal microfilariae periodicity was shown with a peak in numbers between 2100 and 0100 hours. Counts of 7,000 microfilariae per 60 c. mm. were noted. In general there was a greater density of microfilariae in the peripheral blood of female than in the peripheral blood of male monkeys.

In a study to determine the potential vectors in the filarial positve area, over 7,000 mosquitoes of 22 species were collected principally from monkey-baited traps. These were identified, sexed, dissected, and examined for developing filarial larvae. Single developing larva was seen in the thorax of 2 mosquitoes, and in the head of 2 mosquitoes. A single microfilaria was seen in the mesenteron of 2 mosquitoes and in 1 ceratopogonid. In supplementary studies using light trap material, 11 species of *Culicoides* were collected. *Culicoides amamiensis* comprised 82 to 95 percent of the ceratopogonids collected. No filarial larvae were found in over 1,000 *Culicoides* dissected from light trap material. All *Culicoides amamiensis* collected immediately following engorgement from a filarial positive monkey and dissected contained motile, active microfilariae.

From 1962 to 1967, 22 species of arthropods were experimentally exposed to infective blood meals from monkeys with infections of *Macacanema formosana*. The arthropods fall into 5 distinct groups based on the fate of the microfilariae from the peripheral blood: (1) The ceratopogonids, *Culicoides amamiensis*, *Culicoides variipennis*, and to some extent, *Culicoides guttipennis*, ingested a

blood meal containing active, motile microfilariae. The blood meal was digested and the microfilariae made their way to the thoracic muscles to develop. The 3d stage larvae were observed emerging from the proboscis of *Culicoides amamiensis* after 16 days. The remaining groups were not as successful. (2) The argasid tick, *Ornithodoros tartakovskyi*, and the reduviid, *Rhodnius prolixus*, retained the blood meal for at least 24 days, but the microfilariae did not develop. (3) *Stomoxys*, *Chrysops*, and 5 genera (14 species) of culicids took a blood meal containing active, motile microfilariae. However, the microfilariae passed with the blood dejecta from the mosquito or fly in 48 to 120 hours with no development of the microfilariae. (4) *Pedicinus eurygaster* (lice) collected on trapped filarial positive monkeys contained plasma or body fluids but no microfilariae were noted. (5) The 1 species of *Simulium* exposed to a positive monkey would not take a blood meal.

From a review of the climatic, environmental, and ecologic conditions, it is apparent that the simian population has been pushed into the higher elevations. The mountains play a decisive role in determining the climate, rainfall, natural vegetation, history of land settlement and utilization, pattern of settlement, and serve as barriers to both human and primate movements. These factors in turn would affect the simian and arthropod populations.

Although the experimental and circumstantial evidence strongly favors the hypothesis that *Culicoides* is the arthropod involved in the natural transmission of *Macacanema formosana* from monkey to monkey, its acceptance leaves some questions with incomplete answers. The low filarial rate in the insects seems to suggest that it is not an effective vector. However, the very large numbers in which these insects have been shown to congregate about and bite monkeys could compensate for the low infection rate. Thus it would appear from the low infection rate in the observed *Culicoides* that many of the individual insects are not susceptible, but that *Culicoides amamiensis* as a population may be an efficient vector of *Macacanema formosana*.

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INTRODUCTION

The first account (Hsieh, 1961) of filarial parasites in the Taiwan monkey (*Macaca cyclopis*) reported, but did not identify, the microfilariae in the peripheral blood. The adult worms were identified by Schad and Anderson (1963) as *Macacanema formosana*, a new genus and species of the family Onchocercidae, subfamily Dirofilariinae. Preliminary studies by Kim and Bergner (1964) showed that the microfilariae in the peripheral blood of the monkey demonstrated a marked nocturnal periodicity. The purpose of this report is to present the results of the following:

1. A systematic survey of the Taiwan simian population (*Macaca cyclopis*) to determine the distribution and prevalence of filarial parasites.
2. A survey to determine the potential vectors in the filarial positive areas.
3. A study of the development of the filariae in the various suspected intermediate hosts.
4. A study of the biology of the microfilariae in the monkeys.

SECTION I

THE TAIWAN MONKEY, *MACACA CYCLOPIS*

The classificaton by Simpson (1945), generally accepted by American taxonomists, divides the order Primates into the two suborders, Prosimii and Anthropoidea Mivart, 1864. The suborder Anthropoidea classically divided into Platyrrhini and Catarrhini, dating from Hemprich, 1820 and still widely used, was abandoned by Simpson (1931) in favor of dividing it into three superfamilies: the Ceboidea, the Hominoidea and the Cercopithecoidea.

Walker (1964) described the family Cercopithecidae as having 16 genera and 60 species that are found in such varied locations as forests, open regions, rocky areas, and mangrove swamps Both Simpson (1945) and Ellerman and Morrison-Scott (1966) divide the Cercopithecidae into two subfamilies: the Colobinae; and the Ceropithecinae, to which *Papio* and *Macaca* belong.

Ulmer (1960) considers the Genus *Macaca* of the Ceropithecidae as the most successful monkey in the world today. Except for the Barbary macaque, they are of Asiatic distribution, ranging from Afghanistan to the Philippines, and from Java and Timor to 41 degrees north latitude on the main island of Japan. From south to north they demonstrate the principle of reduction of extremities to conserve body heat. At least 11 species of macaques are recognized, indicating their successful position in the primate world. Ellerman and Morrison-Scott (1966) list the following species of *Macaca: assamensis, cyclopis, fuscata, irus, mulatta, nemestrina, radiata, silenus, sinica, speciosa,* and *sylvana. M. sylvana* is the type species. According to Johnson (1967), "Ellerman and Morrison-Scott, 1966, did not include the Celebes, where other species occur: i.e., *M. maurus* and *M. nige*."

The species are distributed as follows: *M. sinica, M. radiata,* and *M. silenus,* are confined to peninsular India and Ceylon; *M. nemestrina* and *M. irus* occur from Burma south-eastward through the Malaysian region; *M. mulatta, M. speciosa,* and *M. assamensis* are roughly Himalayan-Indo-China-Chinese in range; *M. fuscata* from Japan; *M. cyclopis* from Taiwan (Formosa); *M. maurus* and *M. niger* from the Celebes.

Description of *Macaca cyclopis.* In general, the Genus *Macaca* Lacepede, 1799, is described by Allen (1938) as:

> "rather heavy-bodied monkeys, with short stout limbs, and variable tails, usually less than the length of head and body, but sometimes reduced to a mere stump. The nostrils open slit-like and downward. There is a pair of conspicuous callosites on the buttocks. These monkeys have cheek pouches in which food may be temporarily stored. The eyebrow ridges in the skull are very heavy, giving the face a beetling brow; the canines in the males are long, sharp, and strong with a groove on the outer face.

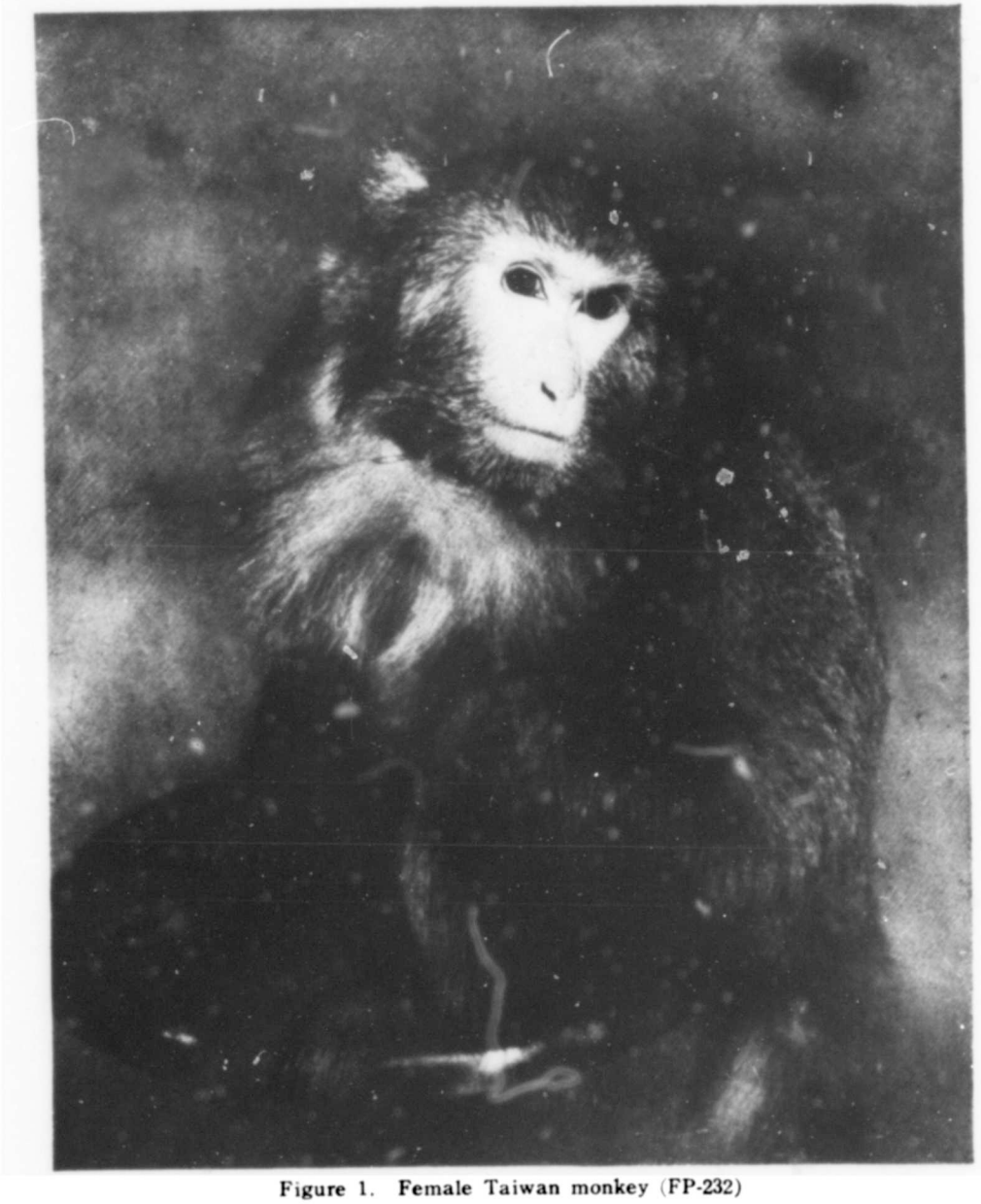


Figure 1. Female Taiwan monkey (FP-232)

> The first and second lower molars show cusps each in two transverse rows, while the third lower molar has a fifth posterior cusp. The tooth formula, as a characteristic of the family is: i.2/2 c.1/1 pm.2/2 m.3/3×2=32

Chen, (1955) described the Taiwan monkey. ***Macaca cyclopis*** Swinhoe, 1862, as above except that:

> "the front limbs are longer than the rear. That it has a simple round stomach and a rather long, tough tail (300mm.). The Taiwan macaque has a round head, flat face, projected forehead, and dark, scarf-shaped moustache. It has long rather soft-wave like hair of dark slate brown color and very thick hair on the limbs which is almost black. The skin of the face and buttocks are light purple-greyish, the belly is white-greyish" (Fig.1)

The Taiwan monkey or the Formosan rock monkey is known as "Yogai, Futton. Nubon, and Rodon" by the various aborigine tribes. Johnson (1967) states that "***Macaca cyclopis***" is the only monkey native to Formosa. It is sufficiently different from other species of ***Macaca*** that occur in Japan, the Philippines, and mainland China to justify current status as a distinct species."

Survey and Identification Procedures

Selection of Survey Area. From July 1962 to May 1964, 277 monkeys were trapped alive throughout Taiwan to determine the prevalence of the filarial parasite, ***Macacanema formosana***. A systematic search for monkeys with this infection was begun in November 1962. Taiwan was divided into 8 geographical districts, using mountain ridge lines which are also Hsien (county) lines as natural boundaries (Fig.2) as follows: Taipei and Taoyuan Hsien, area No. 1; Hsinchu and Miaoli Hsiens, area No. lb; Taichung, Changhua, Nantou, and Yunlin Hsiens, area No, 2; Chiayi, Tainan, and part of Kaohsiung Hsiens, No. 3; Shau-shan (near Kaohsiung city) area No, 4; Pingtung Hsien area No. 5; Taitung and Hualien Hsiens, area No. 6; and I-lan Hsien .area No. 7. Animals originating off the island are listed as area No. 0.

Staff members and hunters in charge were indoctrinated in the use of the compass and topographical grid maps. When an animal was trapped, the location was plotted on the map and the grid vectors logged. Each animal was given a consecutive number, tagged, examined for ectoparasites, given a general physical examination, and examined for blood parasites. Recorded data included the date, hour, location of the collection and the general condition of the animal. Animals were shipped in individual cages to Taipei by any available transportation. A member of the team accompaned each shipment to supervise the care and comfort of the animals.

Distribution of the Monkey. No attempt was made to determine the relative or absolute density of monkeys in an area, between areas, or to correlate the monkeys

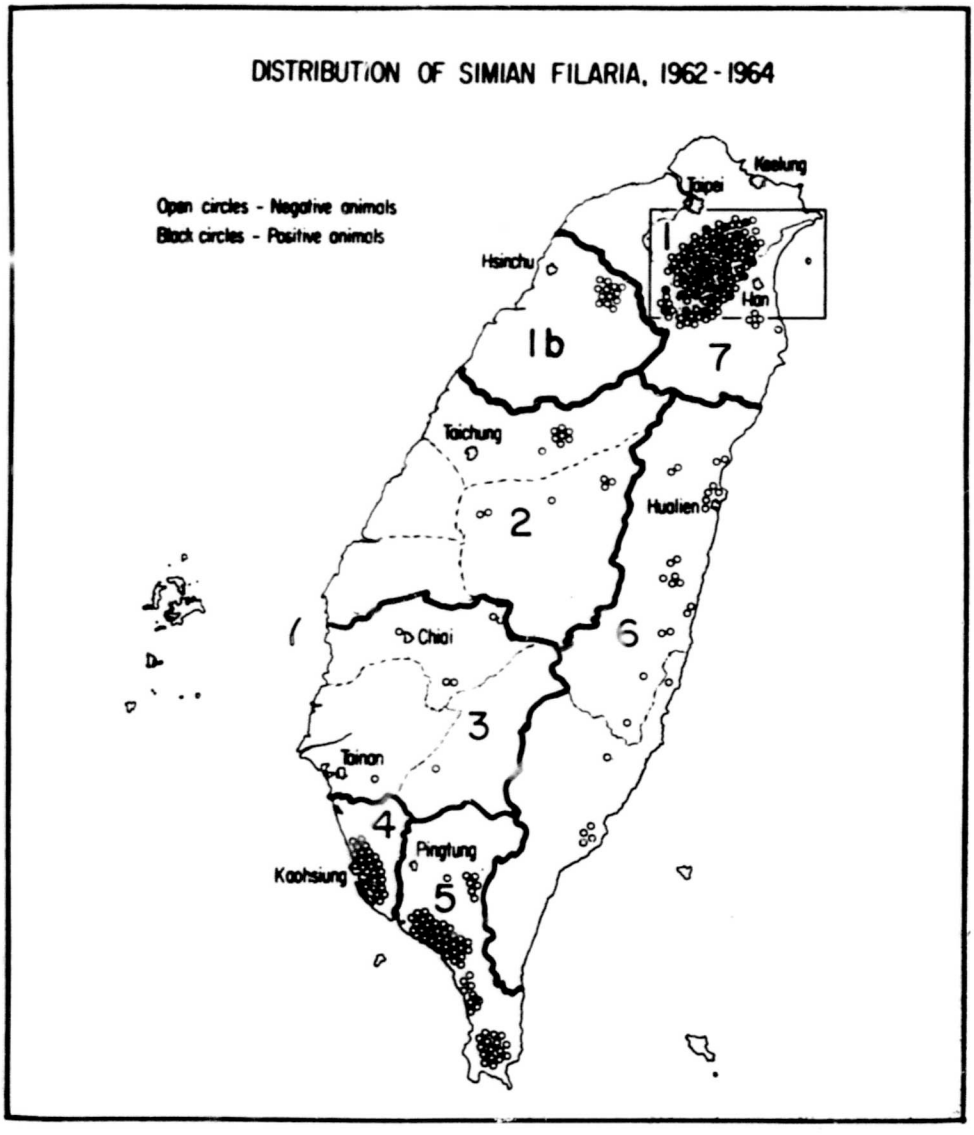


Figure 2. Distribution of Taiwan monkeys trapped 1962-1964 (Each circle represents a monkey trapped).

trapped per man hour. A total of 277 monkeys were trapped in the following areas: 55 in area 1, 19 in lb; 28 in 2; 8 in 3; 24 in 4; 80 in 5; 30 in 6; 20 in 7; and 13 in area 0 (Fig. 2). A close correlation exists between the topography, the type of soil, the kind of forest, and the geographic location of the monkey trapped. In general, the monkey habitat was less than 1000 meters above sea level (Fig. 3), the soil was composed of gray-brown and yellow podsolic or yellow and red young soils (Fig. 4), and the forest was composed of broad-leaved trees (Fig.5). An overlay based on the above limiting factors contained all monkeys trapped. The isolated area No. 4 of Shau-shan (Kaohsiung) along the coast only 6 1/2 by 3 miles in area, meets the same criteria, although it is not shown graphically on the maps presented.

A brief history of the island, its topography, physiographic regions, climatology, the human effects on the basic ecology, and the specific habits and ecology of the monkey have been discussed (Bergner, 1967). For the sake of convenience, the average annual rainfall (Fig. 6), the stages of land settlement (Fig. 7), and the density of population (Fig 8) have been included here.

The horizontal movements of the monkeys tend to be north-south; the central mountain serves as a barrier to east-west movement except at the northern and southern tips of the mountain range. In addition to the central mountain range, the mountain range in a line from I-lan on the east coast to Taichung on the west plain serves as a topographical barrier to north-south movement. Prior to the days of extensive agriculture when the lowlands were still covered by forest, monkeys could move more freely from west to east and from north to south around the periphery of the mountain range.

The monkey from the north is larger and more robust than the monkey of the same age (determined by dental structure, Hurme, 1960) from the southern part of the island. Gradation between the two groups does not appear. The possible explanations might be some isolation of the gene pool with natural selection toward the larger monkey in the northern section has occurred or that the quantity and quality of the available food favors the northern monkey. Infant monkeys from both areas raised on the same diet in the laboratory for 4 years did not show marked differences in size or weight. The lush vegetation that results from greater rainfall and length of the rainy season in the northeastern part of the island is apparent. These factors suggest the difference is nutritional. In reality, over a long period of time, the size differences probably result from both natural selection and nutrition.

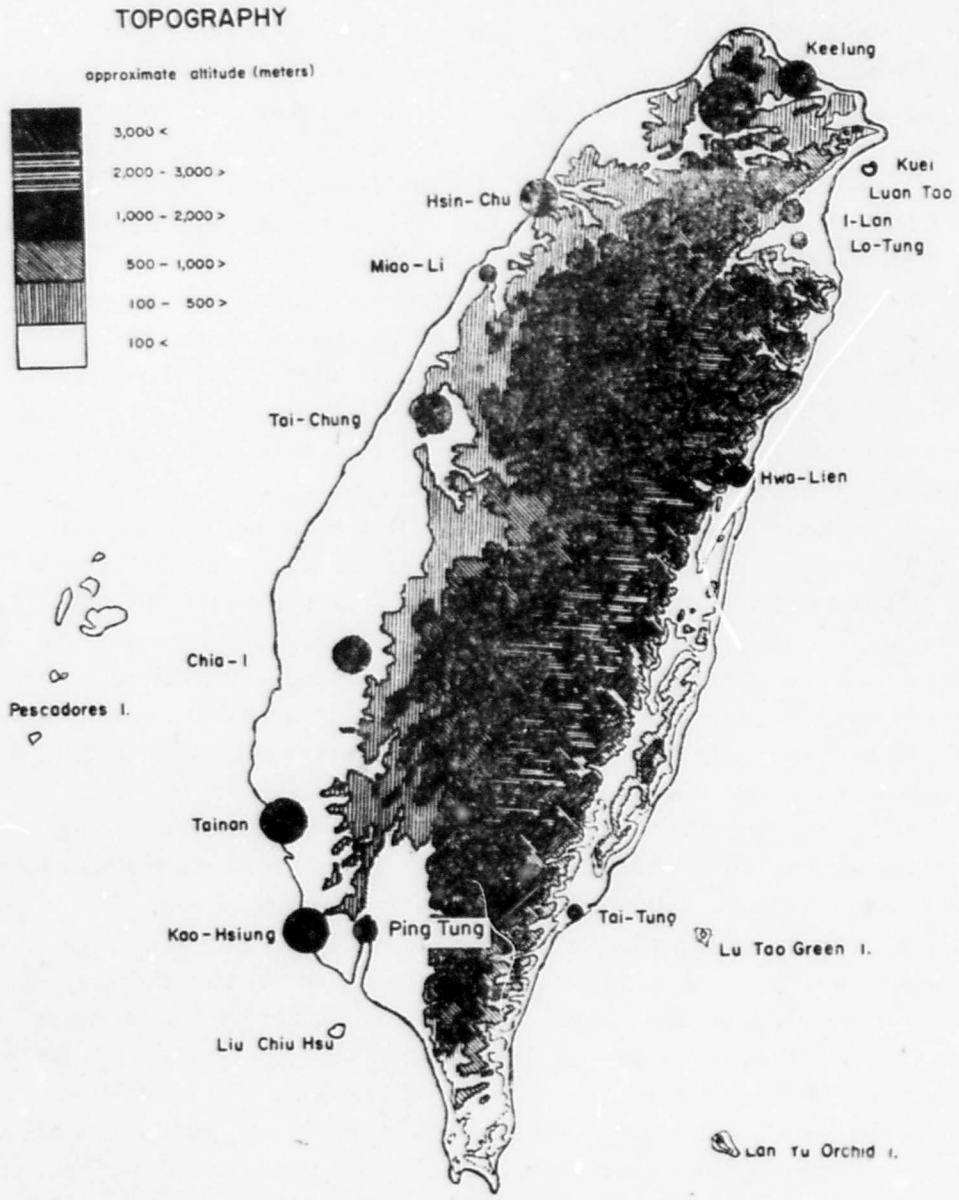


Figure 3. Topographic map of Taiwan showing approximate altitude in meters (after Chen, 1963). (Dark circles indicate relative size of the cities)

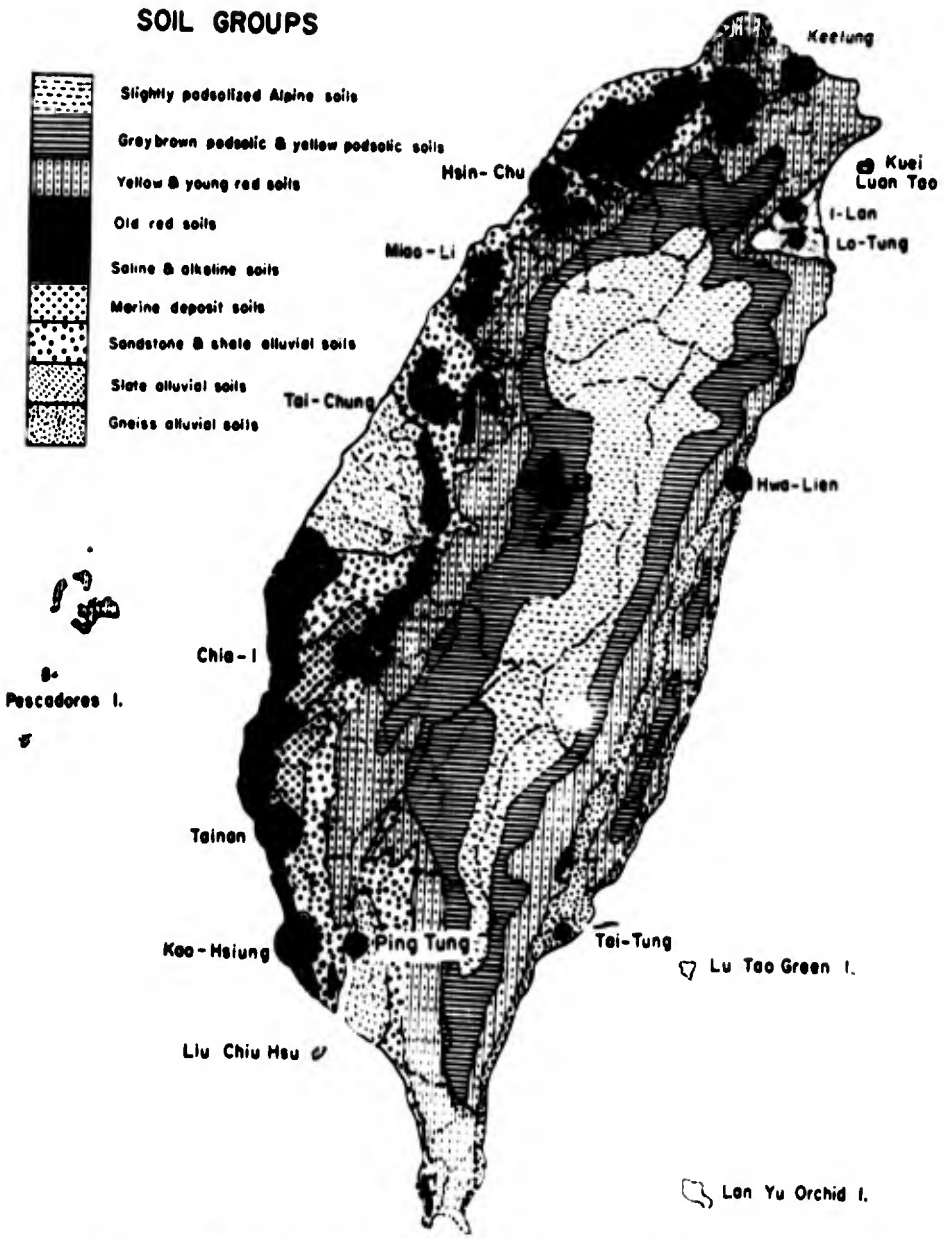


Figure 4. Map of Taiwan showing various soil groups (after Chen, 1963). (Dark circles indicate relative size of the cities).

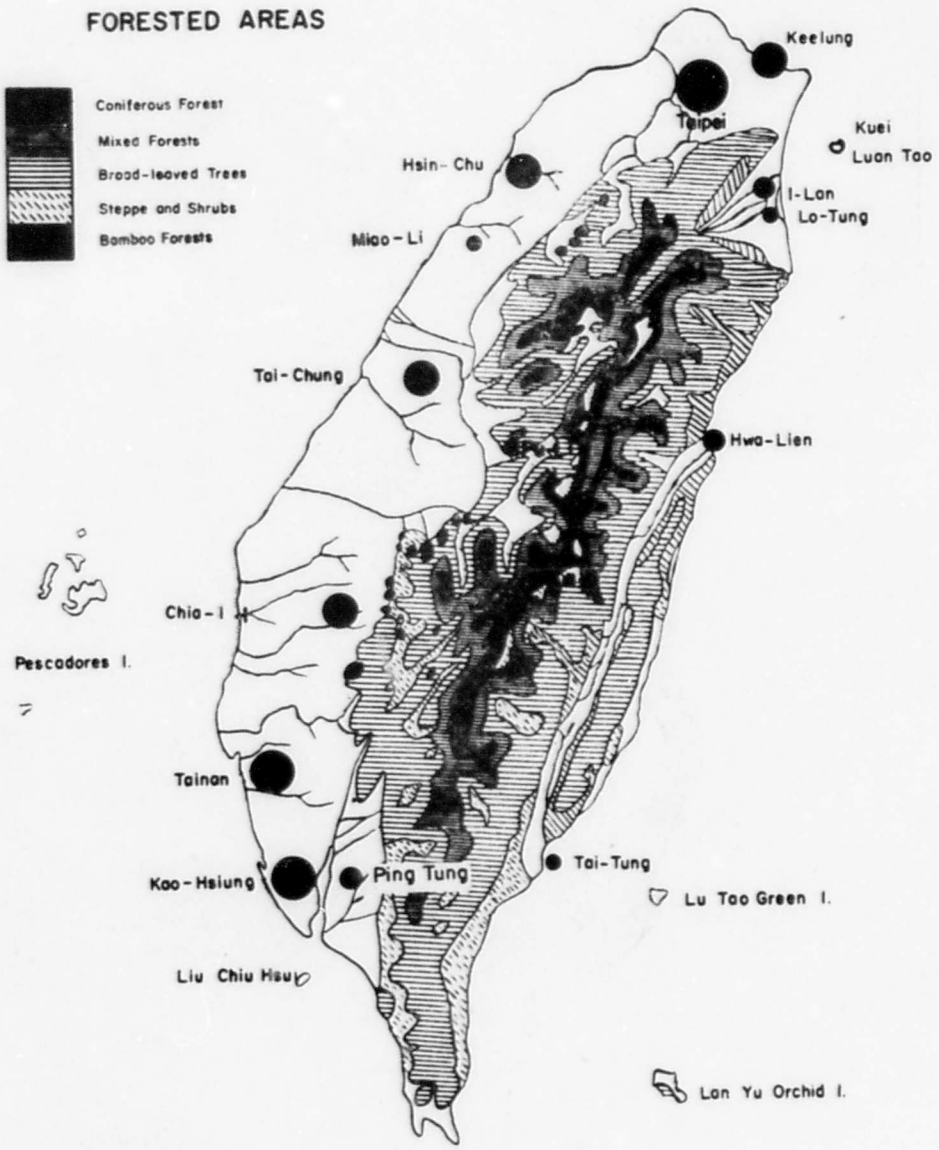


Figure 5. Map of Taiwan showing types of forest areas (after Chen, 1963). (Dark circles indicate relative size of the cities).

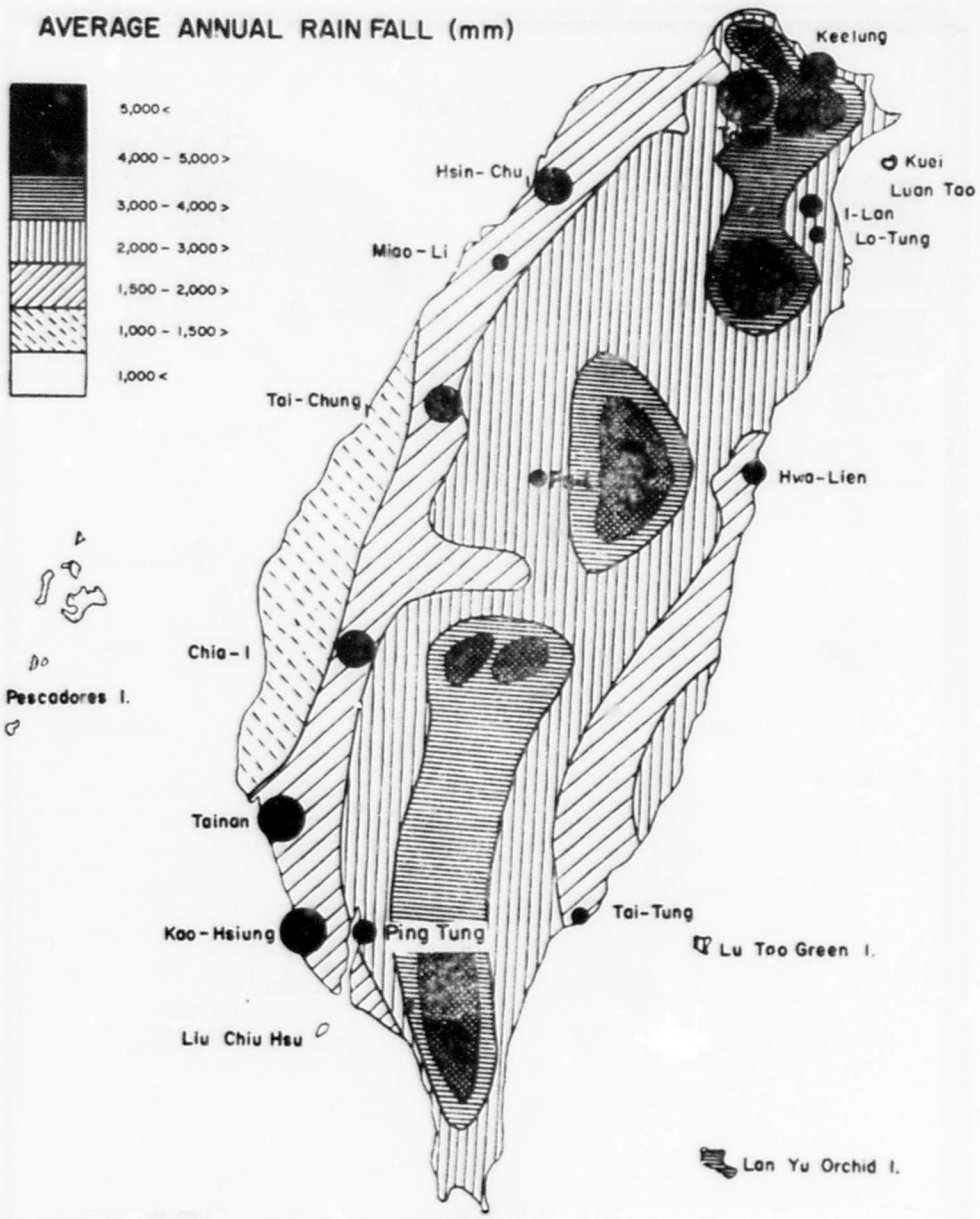


Figure 6. Map of Taiwan showing average annual rainfall in millimeters (after Chen, 1963). (Dark circles indicate relative size of the cities).

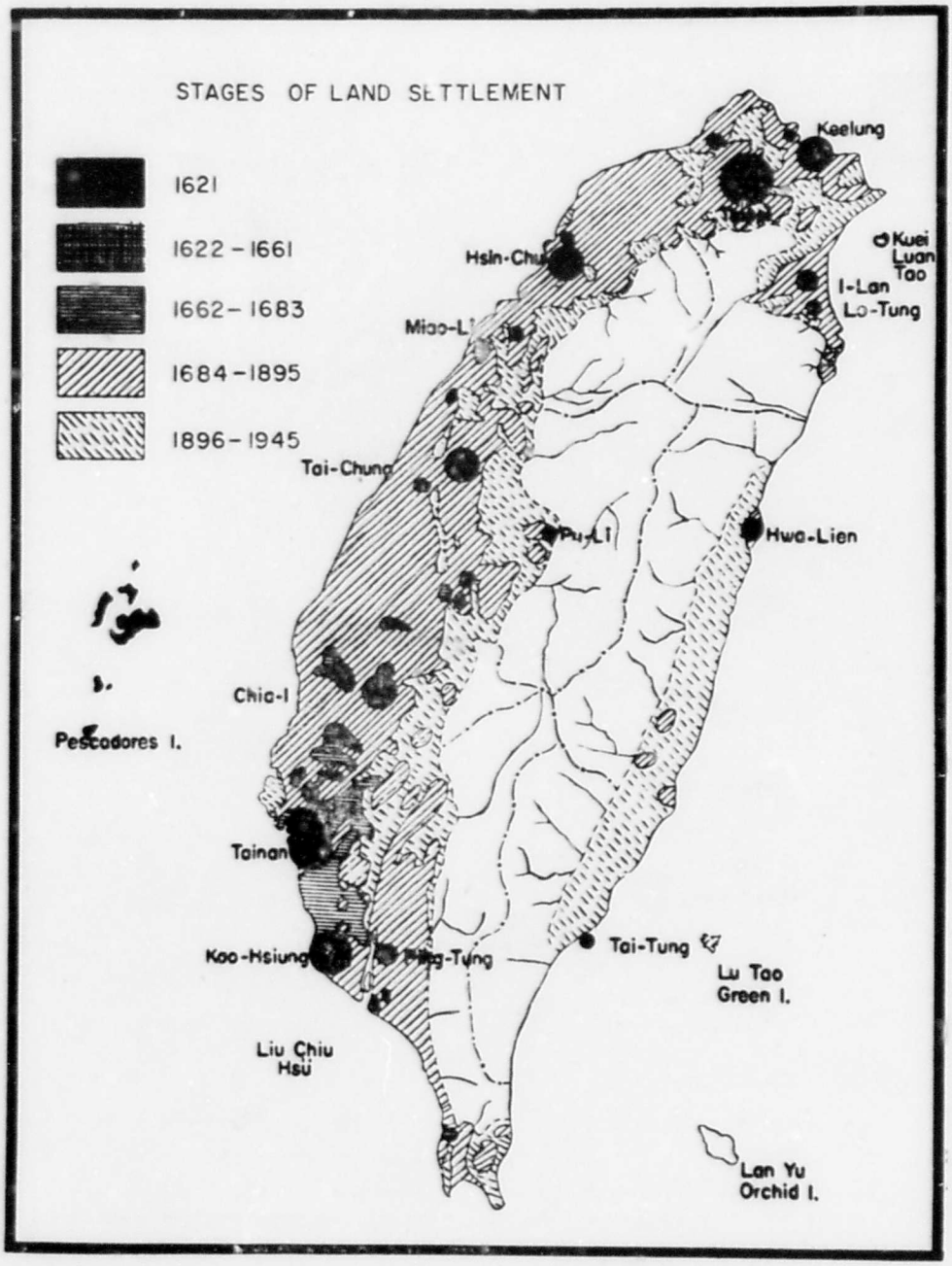


Figure 7. Stages of land settlement in Taiwan (after Chen, 1963). (Dark circles indicate relative size of the cities).

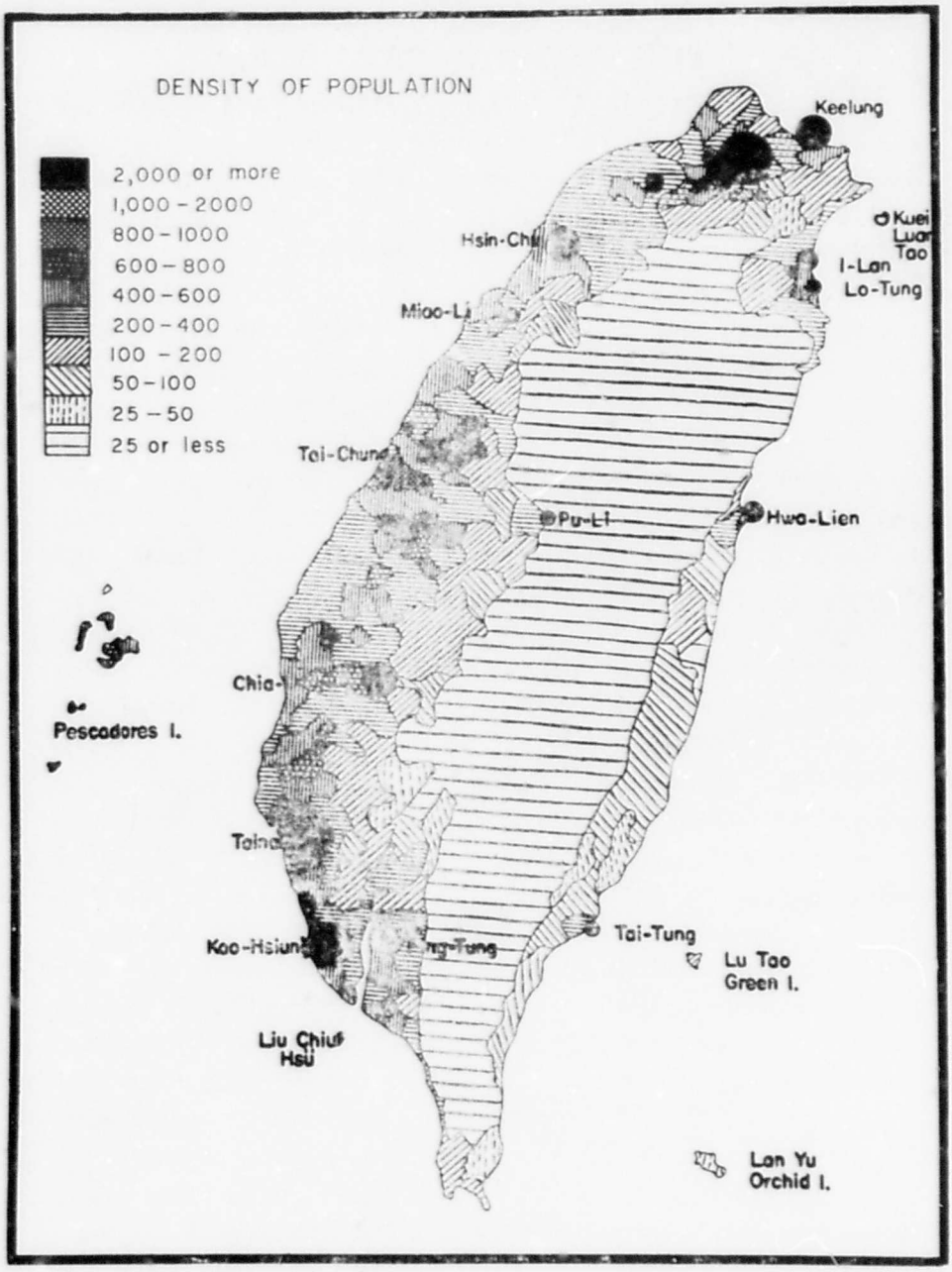


Figure 8. Density of population per sq. km. in Taiwan (after Chen, 1963). (Dark circles indicate relative size of the cities).

SECTION II

THE FILARIAL PARASITE, *MACACANEMA FORMOSANA*

Development of Classification Chabaud and Choquet (1953) stated that the classification of the filariae poses one of the most difficult problems of systematics that can be found in helminthology. This is due, in part, to the fact that the filariae are nematodes that have been profoundly modified by their biology and their adaptation to life in tissues has led to reductions of organs and to such marked convergences that it has been often impossible to determine exactly the origin and the decendants of the different genera of the group. They appear to be essentially polyphyletic. A general history of the classfication of the filariae demonstrates further study is necessary to determine the actual relationships among primate filariae and seems pertinent at this point.

The species of ***Filaria*** were reviewed by Molin (1858), Diesing (1861), and Stossich (1897), but most of the early descriptions are insufficient to relate the worms to those described by later authors. The work of Yorke and Maplestone (1926) on filaria, seldom used anymore, is based on the characteristics of the cuticle.

According to Chabaud and Choquet (1953), the work in Russian, of Skrjabin and Schikhobalova (1936, 1948) is more allied to an alphabetical order card-index file than to a zoological classification. Chabaud and Choquet (1953) state that the divisions between the families and subfamilies are so few and so artificial that the most diverse genera are considered together although very close genera are widely dispersed. Price (1959) and Chabaud and Choquet (1953) state that the classification by Wehr (1935), based on elements that seem to have the best phylogenetic value, and in particular on larval, labial, and cephalic characters, appears to be more satisfactory. Elements especially susceptible to the phenomena of convergence such as the cuticle or the size of the spicules, are used on the contrary, only for generic or specific separations. Anderson (1957), in his description of dipetalonematid nematodes and the problem of their evolution, stated that Wehr's classification of the Filarioidea was the first to distinguish clearly between those filarioids which produce microfilariae and those which produce differentiated larvae. The importance of this contribution, largely ignored by all but a few helminthologists, can scarcely be over-emphasized. Nevertheless, Chabaud and Choquet (1953) state that the Wehr system has been used only by a few exceptional specialists because the author has not included dichotomous keys, and only 46 genera are classified.

Wehr (1935) proposed 4 families under the superfamily Filarioidea: Filariidae Claus, 1885; Desmidocercidae Cram, 1927; Stephanofilaridae, new family; and Dipetalonematidae, new family. The family Dipetalonematidae was divided into 2 new

subfamilies: Dipetalonematinae and Dirofilariinae. Synonyms for the new subfamily Dipetalonematinae by Wehr are Onchocercinae Leiper, 1911; Loainae Yorke and Maplestone, 1926, in part; Setariinae Yorke and Maplestone, 1926, in part.

Chabaud and Choquet (1953) adopted the plan of families as proposed by Wehr and expanded the plan to include genera as well. However, the family Dipetalonematidae has been divided into 6 subfamilies rather than 2 subfamilies as proposed by Wehr. These subfamilies are: the Cardionematinae, with a preanal vulva, defined by Yamaguti (1941); the Oswaldofilariinae, new subfamily parasites of reptiles with pre-equatorial vulva; the Dirofilariinae, with short tails and well-developed caudal fins, as defined by Wehr (1935); the Dipetalonematinae, with tails and decidedly unequal spicules, also defined by Wehr (1935); the Splendidofilariinae, new subfamily, with spicules equal or subequal; and the Onchocercinae, new subfamily, having a short tail and very different spicules.

Webber (1955 a, b) reviewed the filarial parasites of primates, (*Dirofilaria*, *Dipetalonema*, *Loa*, *Protofilaria* and *Parlitomosa*), with the exception of those found exclusively in man and reported over 30 species of nematodes of the suborder (superfamily) Filarioidea. This list is by no means complete nor does it discuss those parasites described since 1955. However, the intent is not to list all the parasites but rather to describe the development of the classification.

Of particular interest is the description of a filarioid worm, *Edesonfilaria malayensis*, from the long-tailed macaque, *Macaca irus*, described by Yeh (1960) and redescribed by Yamaguti and Hayama (1961) (Table 1). Yeh (1960) made no mention of some important features of the worm nor did he express an opinion concerning the systematic position of the genus. Yamaguti and Hayama (1961) although assigning *Edesonfilaria* to the Dirofilariinae stated that this genus may turn out to represent a new subfamily based on the peculiar structure of the esophagus, the unusual length of the left spicule associated with the extensive development of caudal papillae and the excessive length of the unpaired uterus.

Schad and Anderson (1963) described *Macacanema formosana*, n. gen., n. sp., a filaria parasite found in the peritracheal and mandibular regions of the Taiwan monkey, *Macaca cyclopis*. They distinguished *Macacanema formosana* from *Edesonfilaria* by the dimensions and morphology of the left spicule, which is relatively short and complex in *M. formosana* rather than long filiform as in *Edesonfilaria*, by the straight short muscular vagina, the straight short uterine trunk, and the asymmetrical arrangement of the male caudal papillae. The histological structure of the glandular esophagus was described. They placed the parasite in the family Onchocercidae and in the subfamily Dirofiilariinae. The description of the adult worm by Kim and Bergner (1964) compares favorably with the description by Schad and Anderson (1963)

Table 1. Comparison of *Edesonfilaria malayensis* and *Macacanema formosana* (Kim and Bergner, 1964).

Species	*Edesonfilaria malayensis*		*Macacanema formosana*	
Author	*Yeh (1960)*	*Yamaguti (1961)*	*Kim and Bergner (1964)*	
Host	*Macaca irus*	*Macaca irus*	*Macaca cyclopis*	
Location	Peritoneal cavity	Abdominal cavity	Peritracheal and mandibular intermuscular connective tissue	
Locality	Malaya, Siam	Thailand	Wu-lai Taiwan	
Mean Measurements in millimeters:				
Female				(mean)
Length	280-350	250	250-417	(306)
Maximum width	1		1	(1)
Nerve ring	0.2	0.25	0.19	(0.19)
Esophagus:				
anterior part	0.8-1.1		0.82-1.40	(1.04)
posterior part	90	86	57	(57)
Vulva	0.61-1.2	1.2-1.5	0.54-1.21	(0.98)
Tail	0.10		0.10	(0.10)
Male				
Length	124-143	100-160	57-87	(70.41)
Maximum width	0.5-1.0	0.1	0.30-0.63	(0.49)
Nerve ring	0.2	0.2	0.13	(0.13)
Esophagus:				
anterior part	0.6-1.0	0.9-1.0	0.35-0.89	(.61)
posterior part	46-60	55	23-30	(25.50)
Tail	0.06	0.05	0.04	(0.04)
Spicule:				
left spicule	10-11	9.5-10.0	0.53-0.92	(0.68)
right spicule	0.15-1.17	0.15-0.19	0.14-0.17	(0.16)

Necropsy and Histologic Procedures The standard autopsy technic with a "Y" primary incision was used (U.S. Naval Medical School, 1959c). Particular care was taken to separate the skin from the underlying tissues to avoid damage to the adult filarioid worms in the peribrachial and peritracheal subcutaneous and intermuscular tissues. After the skin was separated from the carcass, the connective tissue and muscles were carefully separated with a blunt knife-handle and forceps and examined for adult worms; the body cavity was then opened and examined for adult worms. The organs were removed, examined for parasites with the aid of a dissecting microscope, and representative tissues were taken for histological examination. In certain cases, the adult worm was removed with surrounding tissue

in toto and sectioned.

Proper and immediate fixation is important. In most cases tissues were fixed in 20 volumes of 10 percent buffered formalin for 6 to 12 hours and transferred to fresh fixative prior to examining and sectioning the tissue. Representative sections were processed by the routine paraffin technic, sections cut and then stained with the routine hematoxylin and eosin stain.

Collection, Fixation, Preservation, and Examination of Adult Parasites When live adult filarioids were found following the necropsy procedure described in the previous section (Figs. 9, 10), they were gently removed by dropping warm physiological saline on the surrounding tissue and rolling the parasite around an applicator stick, at the same time cutting the worm free from the surrounding connective tissue with iris scissors. The worms were further relaxed by placing them singly into steaming saline, killed by immersion into hot water and transferred to FAAG (5 ml. commercial formalin, 2 ml. glacial acetic acid, 75 ml. of 95 percent ethanol, and 5 ml. of glycerine, q. s. to 100 ml. with disitilled water). After 6 to 12 hours, the worms were transferred to fresh FAAG and later transferred to and stored in 70 percent ethanol with 5 percent glycerine. Phenol-alcohol (4:1) was used as a clearing agent prior to examination of the adults. Identity confirmation of the adult filaria was made by Mrs. Maybelle Chitwood of the Beltsville Parasitological Laboratory, Agricultural Research Service of the U.S. Department of Agriculture. Other adult helminths were deposited with Drs. Robert E. Kuntz and Betty June Myers at the Southwest Foundation for Research and Education, San Antonio, Texas.

Parasite Prepatent Period The prepatent period of *Macacanema formosana* has not been determined. Little is known of the prepatent periods of other filarioid worms because there have been few attempts to transmit them experimentally or to collect epizoological information which might provide an estimate. *Dirofilaria immitis* is said to have a prepatent period of some 8 to 9 months (Bancroft, 1904, Webber *et al.*, 1955b). Chardome and Peel (1951) have estimated that the prepatent period of *Dipetalonema streptocerca* is about 9 to 12 months. They also reported microfilariae of *Loa loa* in the blood of 1-year-old child. In contrast, Anderson (1956) and Robinson (1955) have indicated that some avian filarioids have an 8 week or shorter prepatent period. The prepatent period of *Macacanema formosana* is probably greater than 6 months, probably 6 to 12 months.

Collection, Preparation, and Examination of Blood Specimens In order to determine the prevalence rate of *Macacanema formosana* in the Taiwan monkey, the microfilaria prevalence rate was selected as the main criteria and method of choice. The monkey must be or have been infected with the adult gravid parasites to have microfilariae of *Macacanema formosana* in the peripheral blood. The adult filariae are found in the peritracheal and submandibular tissues of the

Figure 9. Autopsy of Taiwan monkey showing adult filaria in the peritracheal subcutaneous region

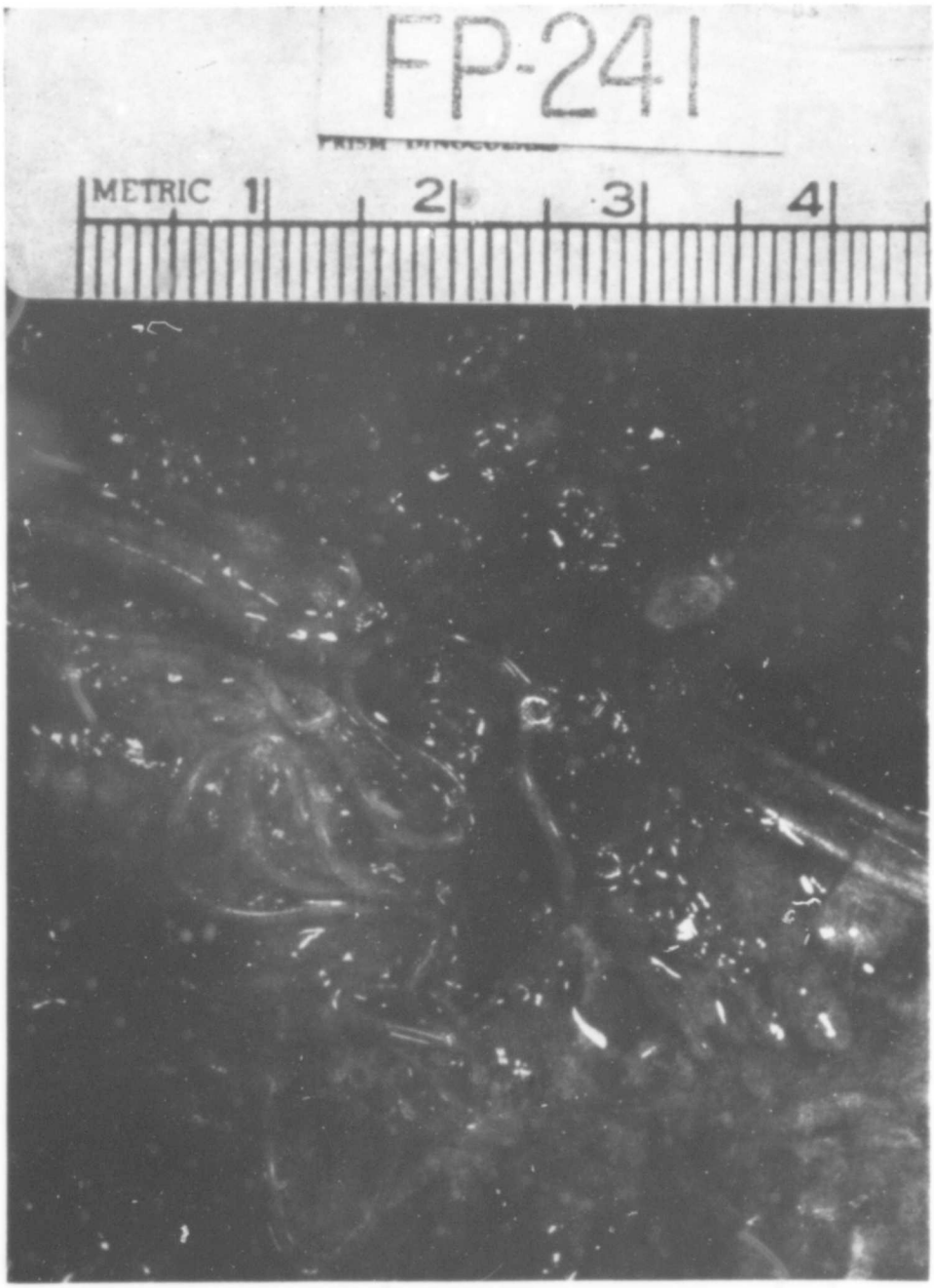


Figure 10. Adult filaria in the peritracheal region of a Taiwan monkey

monkey. Outward physical signs which are attributed to the blockage of the lymphatics in humans with *Wuchereria bancrofti* are lacking in the monkey, as are other clinical signs. The use of present filarial antigens for skin testing are non-specific. A 2d criteria to supplement the microfilarial prevalence rate is, of course, the recovery of adult worms at necropsy.

A total sample of 60 c.mm. of blood, consisting of 3 thick smears containing 20 c. mm. of blood each plus a thin smear, was drawn aseptically from the upper margin of the ear or from the saphenous leg vein of the monkey into disposable micro-hematocrit capillary tubes marked and calibrated to contain 20 c.mm. volume against a Sahli blood pipette. Each 20 c.mm. of blood was discharged onto a clean glass slide and marked with the consecutive monkey number, date and hour of the preparation. In the field, the thick blood smears were examined immediately for microfilarial motility under the low (10×10) and high (10×44) power of a compound microscope and later confirmed by the routine staining methods. The blood was fixed to the slide, air-dried overnight at 25°C. or for 1 hour in a 37°C. drying box. The specimen was dehemoglobinized in buffered water (pH. 6.8-7.0), fixed in absolute methyl alcohol and stained with Giemsa. Occasionally Harris, Delafield's or Haedenhain's hematoxylin was used for special examination, as described in the navy manual (U.S. Naval Medical School, 1959a). Measurement of microfilaria described in the peripheral blood of *Macaca cyclopis* is summarized (Table 2). The thin smears were air-dried, fixed in absolute methyl alcohol and stained with Giemsa. All smears were coverglassed utilizing Permount mounting media and examined under a compound microscope at 100 magnifications. Confirmation was made under higher magnification. Microfilariae were counted with the aid of a squared eye piece and the number recorded for each sample. In each case the slide was read by more than 1 person, the discrepancies noted and corrected. Each slide was examined for 15 minutes before it was classified as "no parasites noted." Variation in the numbers of microfilariae in the 3 smears rarely exceeded 10 percent from the 3-smear averages. In all cases, at least 2 samples were taken on the initial examination, a diurnal sample between 0900 and 1600 hours, and a nocturnal sample between 2100 and 0100 hours. In periodicity studies the blood samples were drawn every 2 or 4 hours beginning at 0900 for a 24-, 48-, or 72-hour period. Monkeys on which the blood smears had been classified as "no parasites noted" were retested by the Knott (1939) concentration method.

The Results of Blood Examination The prevalence of blood parasites in the Taiwan monkey, *Macaca cyclopis*, by sex and geographical area is shown in Table 3. All monkeys infected with the filarial parasite, *Macacanema formosana* were trapped in area 1, Taipei and Taoyuan Hsiens (Figs. 11, 12). Of the 55 animals trapped in this area, 23 (42 percent) were positive for microfilariae in the perpheral blood. Certainly, the 42 percent filarial positive is a conservative figure since it does not

Figure 11. Distribution of simian filaria, by area, 1962-1964 (Insert area No. 1)

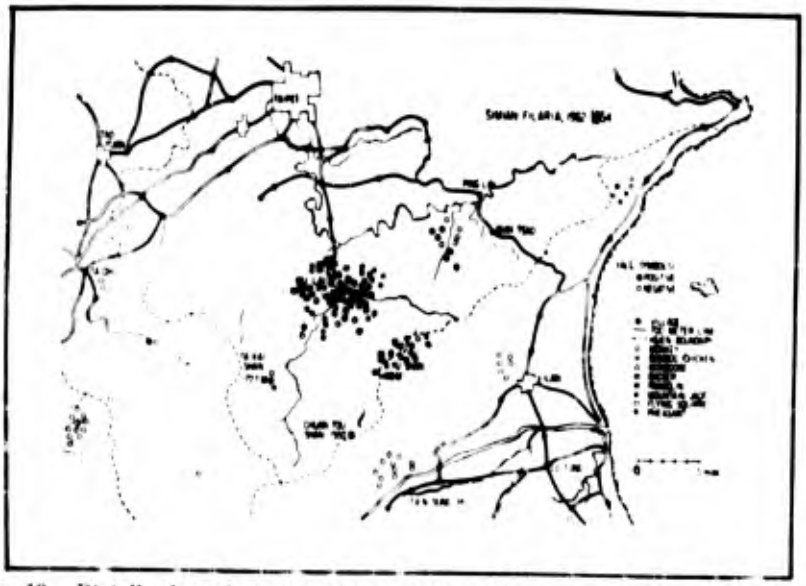


Figure 12. Distribution of simian filaria, 1962-1964 (Insert area No. 1, enlarged)

Figure 13. Distribution of simian "malaria", by area, 1962-1964 (Insert area No. 1)

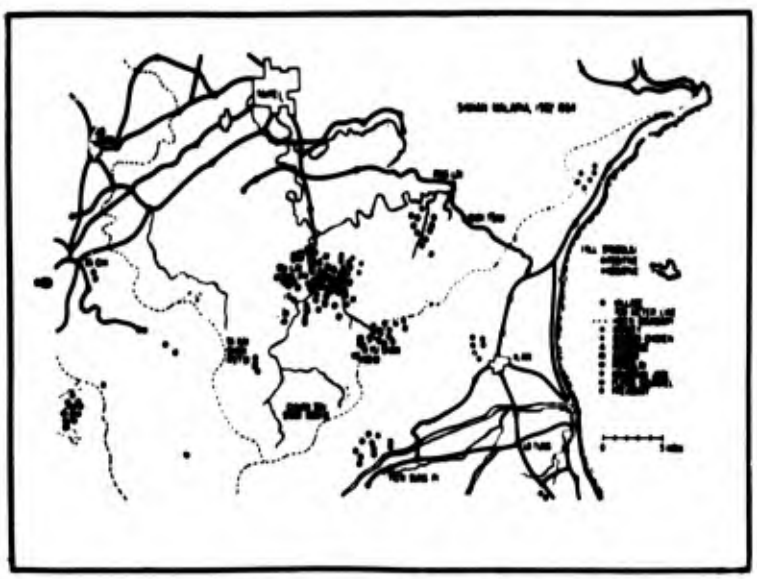


Figure 14. Distribution of simian "malaria", 1962-1964 (Insert area No. 1, enlarged)

Table 2. Measurements of microfilariae described in the peripheral blood of *Macaca cyclopis* (Kim and Bergner, 1964).

Stain	Kim and Bergner (1964)		Hsieh (1961)
	Giemsa (thick) Length (range)	Azur II (vital) Length (range)	Giemsa (thin) Length (range)
Total length (microns)	170.71(151.19-180.08	217.91(209.44-230.72	151.88(137-179)
Width	3.00(2.24-3.36)	4.48	2.97(2.7-3.4)
Percent distance from anterior end to:			
First nucleus of nuclear column	1.97(1.59-2.48)	2.26(1.94-3.03)	1.9(1.43-2.28)
Nerve ring	20.18(19.06-22.4)	19.38(16.50-21.67)	20.08(18.57-21.20)
Excretory pore	31.87(29.30-35.87)	31.09(23.12-33.99)	28.91(25.97-30.72)
G_1 cell	63.53(58.70-67.98)	63.09(58.54-67.53)	67.53(65.60-69.50)
Anal pore	80.91(79.63-82.03)	80.49(77.72-83.24)	80.30(78.83-81.27)
Percent length from posterior end to:			
Last nucleus of nuclear column	3.03(1.91-4.48)	5.03(4.66-6.09)	3.97(3.42-4.83)
Number of examined microfilariae	10	10	6

take into account single sex infections, infections in which the female was not gravid, infections in which the worms had not yet matured, and infections in which the number of circulating microfilariae had not reached a detectable level.

Of the 277 monkeys examined from November 1962 to May 1964, 71 (26 percent) were positive for "malaria"-like organisms, a term which in this paper may include *Plasmodium cynomolgi*, *P. inui*, *P. knowlesi*, *Hepatocystis taiwanensis*, and *Plasmodium sp.* unknown. "Malaria" was distributed rather evenly in areas 1, 3, 5, 6, and 7, but it was not found in monkeys in areas 1b, 2 and 4 (Figs. 13, 14). Areas 1b, 2, and 4 are on the "dry" side of the central mountain range, but so are areas 3 and 5.

The prevalence of "malaria" and filaria was almost 2 times greater in female monkeys than in male monkeys (Table 3). There may be several reasons for this trend. The author's general impression is that as infants, the male and female monkey have the same relative exposure to both malaria and filaria. During this time the infant remains rather close to the mother monkey, but as the male matures it begins to move to the periphery of the group. The probability of the hematophagous arthropod being attracted to the lone monkey is less than to that of the larger group, which in the case of *Macaca cyclopis* is generally composed of the adult females, their young, and the dominant male. The higher prevalence of malaria in the female holds true in each area surveyed

Theories on Microfilariae Periodicity In 1879, Manson found that the micro-

Table 3. Prevalence of simian blood parasites by sex and area, Taiwan, 1962-1964.

Monkeys	Survey Area (See Figs. 11, 13) 1	1b	2	3	4	5	6	7	0	Total
No. of Males	25	6	13	1	16	23	7	6	5	102
Positive for malaria only	3	—	—	—	—	5	—	1	—	9
Positive for filaria only	2	—	—	—	—	—	—	—	—	2
Positive for both	5	—	—	—	—	—	—	—	—	5
No. of Females	30	12	15	4	8	37	9	14	7	136
Positive for malaria only	6	—	—	1	—	9	2	8	—	26
Positive for filaria only	5	—	—	—	—	—	—	—	—	5
Positive for both	11	—	—	—	—	—	—	—	—	11
No. of monkey's sex undetermined	—	1	—	3	—	20	14	—	1	39
Positive for malaria only	—	—	—	3	—	4	13	—	—	29
Positive for filaria only	—	—	—	—	—	—	—	—	—	—
Total No. of monkeys	55	19	28	8	24	80	30	20	13	277
Positive for malaria only	9	—	—	4	—	18	15	9	—	55
Positive for filaria only	7	—	—	—	—	—	—	—	—	7
Positive for both	16	—	—	—	—	—	—	—	—	16

filariae of *Wuchereria bancrofti* were numerous in the blood of infected patients during the night but they become rare or absent during the day. This phenomenon of alternate increase and decrease in number according to a 24-hour cycle is known as the periodicity of microfilariae. Manson (1879) considered the parturition of the microfilariae a continuous process. For many years it has been known that the circadian cycle of the microfilariae is influenced by the host (Mc Kenzie, 1882) and that a sustained change in the cycle of the host produces a corresponding change in the cycle of the parasite. This observation confirmed by Manson (1882), York and Blacklock (1917), and by others listed in Fulleborn (1929), have indicated that the cycle of microfilariae is influenced by the waking and sleeping activity of the host and not the day and night cycle; in the raccoon the cycles coincide. In most host-parasite combinations the maximal filarial count in the peripheral blood coincides with the period of inactivity of the host. Hawking (1962a) gives a general rule "that the maximum of the microfilaria count is oriented according to the activity of the vector and not according to that of the host."

Hawking and Thurston (1951b) reviewed various hypotheses to explain the production of microfilariae periodicity. One proposed by Myers (1881 and 1886) was

that parturition was continuous, but the microfilariae remained in the lymphatics for 12 to 24 hours and then entered the blood system at the same time, causing the rise in the number of microfilaria; the microfilariae then dissolved and were found dead by noon of the next day, thus explaining the fail in the number of microfilariae.

Lane (1929) revised Myers' theory and postulated "that periodicity must be due to simultaneous parturitions once in 24 hours by the mother worms, the young being destroyed each day" and supported it by histological examination of gravid worms. Harley (1932) suggested a specific chemotaxis acting on the microfilariae caused them to migrate towards the saliva of the insect host. Various questionable theories on the mechanisms of microfilarial periodicity have been reviewed in detail by Lane (1948).

Wong (1964) working with ***Brugia pahangi*** and ***Dirofilaria immitis*** in dogs supported the view that the maintenance of stable populations of microfilariae is a manifestation of successful inter-adaptation between the filarial parasite and its hosts. Previous reports on the transfusion of microfilariae have been reviewed by Hawking and Thurston (1951a) and Hawking (1953) in which they indicated that the presence of adult worms was not needed for microfilarial periodicity. According to Hawking (1962b) "the periodic cycle affects most of the body of man and higher animals.....that is.....during the night the temperature falls, the carbon dioxide pressure rises, the oxygen pressure falls, the body becomes more acid, the kidney secretes less water and chlorides, the adrenal is less active, and etc."

Hawking, Worms, and Walker (1965) transfused microfilariae of ***Edesonfilaria malayensis*** into monkeys at different phases of the circadian rhythm. In short, the transfused microfilariae accepted the cycle of the recipient and they concluded that "probably each microfilaria has an endogenous cycle.....presumably dominated and synchronized (entrained) by unknown stimuli from the circadian cycle of the host." According to this view the behavior of the microfilaria at a specific time depends on 2 factors: one, stimuli supplied by the conditions in the host, two, the experience of the microfilaria during the previous 12 hours. When man or animal is made to reverse his routine so that he sleeps by day and moves about by night, the periodicity of the microfilariae alters accordingly (Hawking, 1962a).

Microfilarial Periodicity of Macacanema formosana Twenty-five monkeys trapped in northern Taiwan from 1962 to 1964 were found positive for the filarial parasite ***Macacanema formosana.*** No microfilariae, only adult worms were found in 2 of these monkeys. The nocturnal periodicity of this parasite was first described by Kim and Bergner (1964 in a preliminary study on a single animal). The individual microfilarial counts made on 17 of the 25 animals on 39 occasion by various technics (ear, tail, saphenous vein, and finger puncture) are given (Table 4).

Blood samples for microfilariae counts were drawn every 2 or 4 hours for a period of 24, 48, or 72 hours. The monkeys were in good general condition and

Table 4. *Macacanema formosana* microfilariae counts in 60 cubic millimeters of blood from 17 *Macaca cyclopis*, 1962-1964.

				Time in hours						
FP#	Sex	Date		0900	1300	1700	2100	0100	0500	0900
23	F	10 Dec 62		4	13	133	261	369	135	4
		12 Dec 62		7	3	99	193	139	69	7
		14 Dec 62		18	36	118	330	346	145	18
		24 Sept 63		5	3	27	180	148	149	5
		14 Oct 63	Ear	1	2	6	70	116	135	4
			Tail	2	3	5	103	102	114	1
			Finger	2	1	6	54	104	76	1
241	F	6 March 63		12	9	14	136	179	152	12
		8 March 63		7	6	149	257	255	139	7
		11 March 63		6	9	56	219	214	106	6
320	F	16 Sept 63		1	1	1	4	2	2	1
		5 May 64		1	0	1	0	0	0	0
322	F	16 Sept 63		65	183	325	414	298	243	42
388	F	20 Sept 63		232	289	1034	2516	3195	2975	202
		5 May 63		145	308	4124	5360	3830	294	83
339	F	5 Sept 63		1679	1760	4018	5127	6949	3998	2076
		18 Feb 64		1	0	0	1	1	1	0
		5 May 64		9	13	15	22	18	10	3
392	M	24 July 63		14	27	620	2056	1730	1022	15
		23 July 63	Ear	20	20	443	1436	2045	1613	20
			Vein	16	24	468	1824	1585	1457	16
		25 July 63		15	39	1843	2217	1926	31	14
		5 May 64		219	272	1339	2391	1267	440	89
396	F	20 Sept 63		0	1	1	15	15	5	0
		5 May 64		0	0	0	0	0	0	0
414	F	24 Sept 63		560	335	534	1925	2118	1501	276
		5 May 64		610	562	4641	6308	4102	2580	507
468	F	19 Nov 63		0	2	40	67	68	36	2
		6 May 64		14	1	16	80	59	37	2
472	M	26 Nov 63		23	213	107	667	740	255	55
		6 May 64		41	82	303	899	1000	326	64
473	M	26 Nov 63		3	14	120	77	56	10	10
477	F	3 Jan 64		8	7	14	201	230	173	8
481	M	3 Jan 64		6	2	19	64	71	48	2
		15 May 64		23	18	202	588	377	251	10
483	F	3 Jan 64		273	103	823	1716	1504	820	85
		18 Feb 64		150	141	978	1359	1221	511	111
		15 May 64		35	14	173	589	400	51	17
486	F	3 Jan 64		163	119	1152	2843	2484	1088	345
		18 Feb 64		112	77	315	1160	967	327	122
487	F	3 Jan 64		19	5	46	146	195	43	4
		18 Feb 64		5	6	33	93	59	27	14
		15 May 64		5	4	22	135	62	22	3

presented no evidence of infection or debility. The light in the animal facilities was not controlled but approximated a 14:10 hour cycle, light from 0500 to 1900, dark from 1900 to 0500. The counts are expressed as the percentage of the total 24-hour count (Figs. 15-20), or as microfilariae per 60 c. mm. of blood (Fig. 21).

On several occasions the examination of microfilariae periodicity was extended for 3 consecutive days. Generally, microfilariae density on each day follows the same nocturnal curve but the slope of the curve rose more abruptly in the 1300 to 1700 period as the experiment continued. The peak of the curve shifted to the left (Fig. 15). The monkeys in this case were disturbed or awakened at 4 intervals with an accompanying loss of sleep over a period of 3 days. This shift in the curve suggests a causal relationship with fatigue and a change in the sleeping pattern.

In the initial periodicity studies, blood samples were obtained at the same time from the vessels of the ear, tail, finger, and leg veins of the monkey for comparison. Finger puncture proved unsatisfactory because fat oozed from the fat pads in the monkeys' finger tips causing a dilution error. The periodicity by these various technics follow the same acute nocturnal pattern, but in samples taken from the larger blood vessels, e.g., the saphenous leg vein as opposed to the vein in the upper margin of the monkey's ear, a greater number of microfilariae appear earlier, in the 1700 to 2100 period (Fig. 17). The ear technic proved to be the easiest method and was used as the standard in all periodicity studies.

The peak shift of 4 hours to the left in 1 monkey that maintained approximately the same total microfilariae count for a period of a year is noted (Fig. 16), but it can not be said that this shift is related to varying habits or physiology in the monkey that would cue the microfilariae because studies were not done to compare monthly or seasonal rhythms or shifts.

The possibility that the total count or density of microfilariae may affect the percentage of microfilariae that would remain in circulation at any particular time was considered. The periodicity counts of microfilariae in animals in which the total 24-hour count had increased (Fig. 18), decreased (Fig. 19), remained the same (Fig. 16) over a period of year, as well as the periodicity counts at different densities (Fig. 20) are shown. A first impression was that at the higher density level (Figs. 19, 20), the slope of the curve was less abrupt when a greater percentage of the microfilariae remained in the general circulation but this did not always hold true (Fig. 18). These studies were conducted at various times throughout the year and monthly and seasonal shifts were not related.

It is shown that the periodicity of microfilariae is markedly nocturnal (Fig. 21). Relatively few microfilariae were found in the diurnal blood films. The microfilariae peaks occurred between 2100 and 0100 hours. Nocturnal counts were as high as 7,000 microfilariae per 60 c. mm. In general, a greater number of female monkeys were infected with *Macacanma formosana*, and those that were infected

demonstrated a greater density of the microfilariae in the peripheral blood. Whether this difference in prevalence or density is a hormonal situation related to the sex of the monkey or related to the difference in rate of exposure under natural conditions could be determined in controlled experiments in the laboratory with clean monkeys challenged with ***Macacanema formosana***.

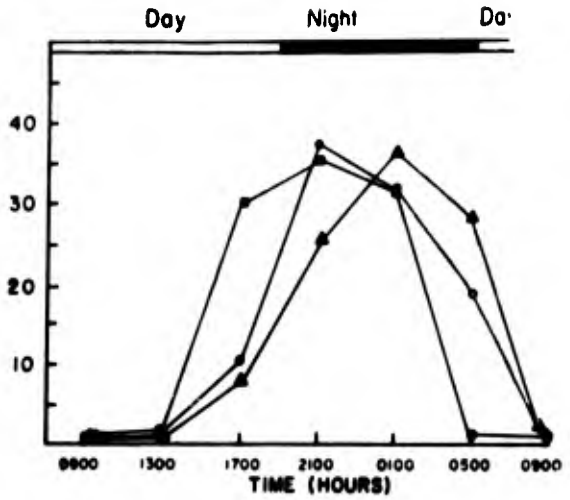


Figure 15. Periodicity of microfilariae of *Macacanema formosana* in *Macaca cyclopis* (1962-1964), on three consecutive days

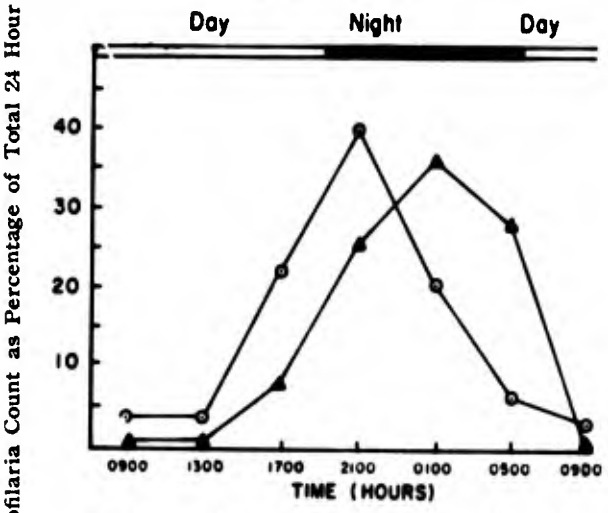


Figure 16. Periodicity of microfilariae fo *Macacanema formosana* in *Macaca cyclopis* over a period of time

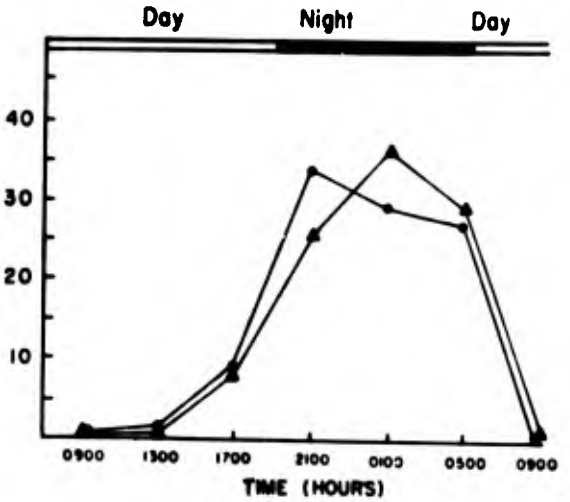


Figure 17. Periodicity of microfilariae of *Macacanema formosana* in *Macaca cyclopis* by two technics

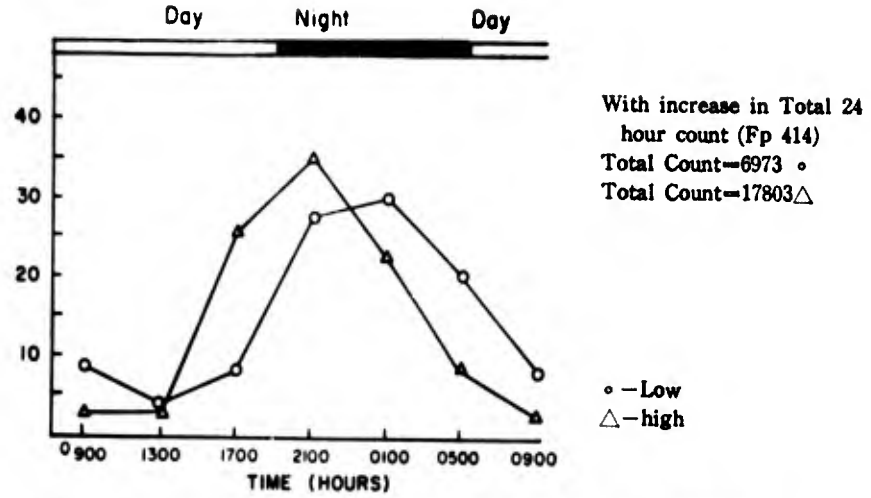
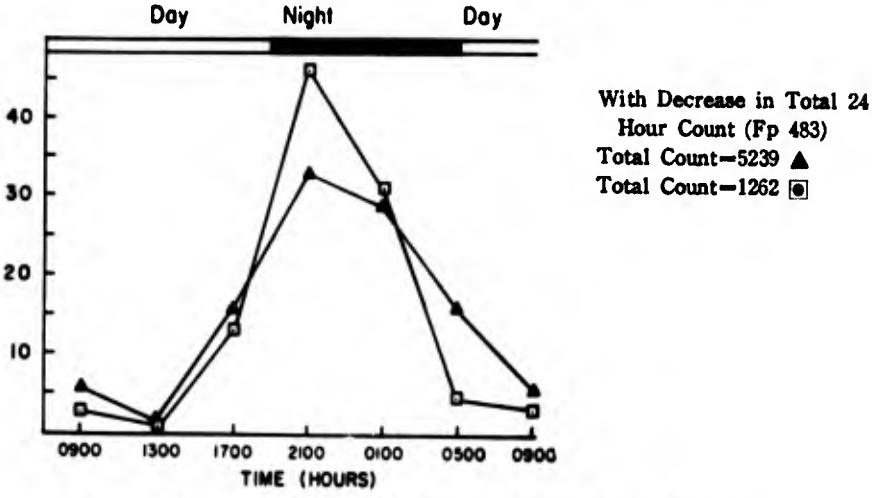


Figure 18. Periodicity of microfilariae of *Macacanema formosana* in *Macaca cyclopis* with increase in total 24 hour count



Microfilaria Count as Percentage of Total 24 Hour Count

Figure 19. Periodicity of microfilariae of *Macacanema formosana* in *Macaca cyclopis* with decrease in total 24 hour count

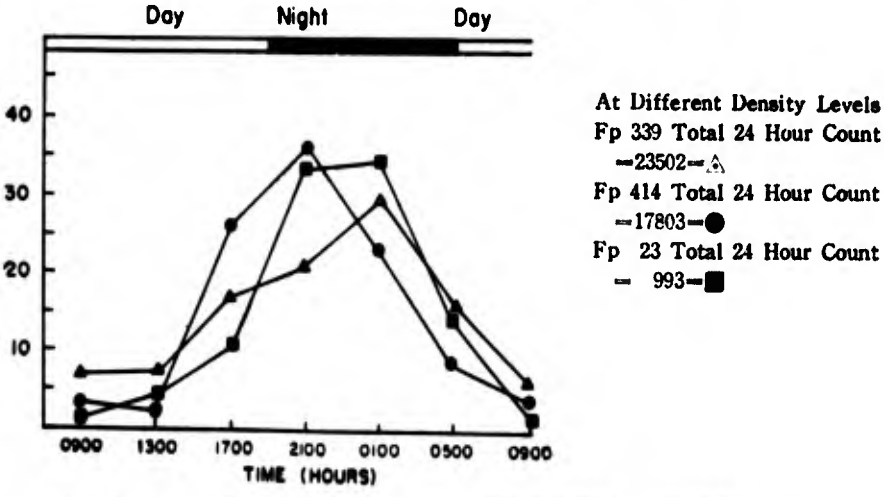


Figure 20. Periodicity of microfilariae of *Macacanema formosana* in *Macaca cyclopis* at different density levels

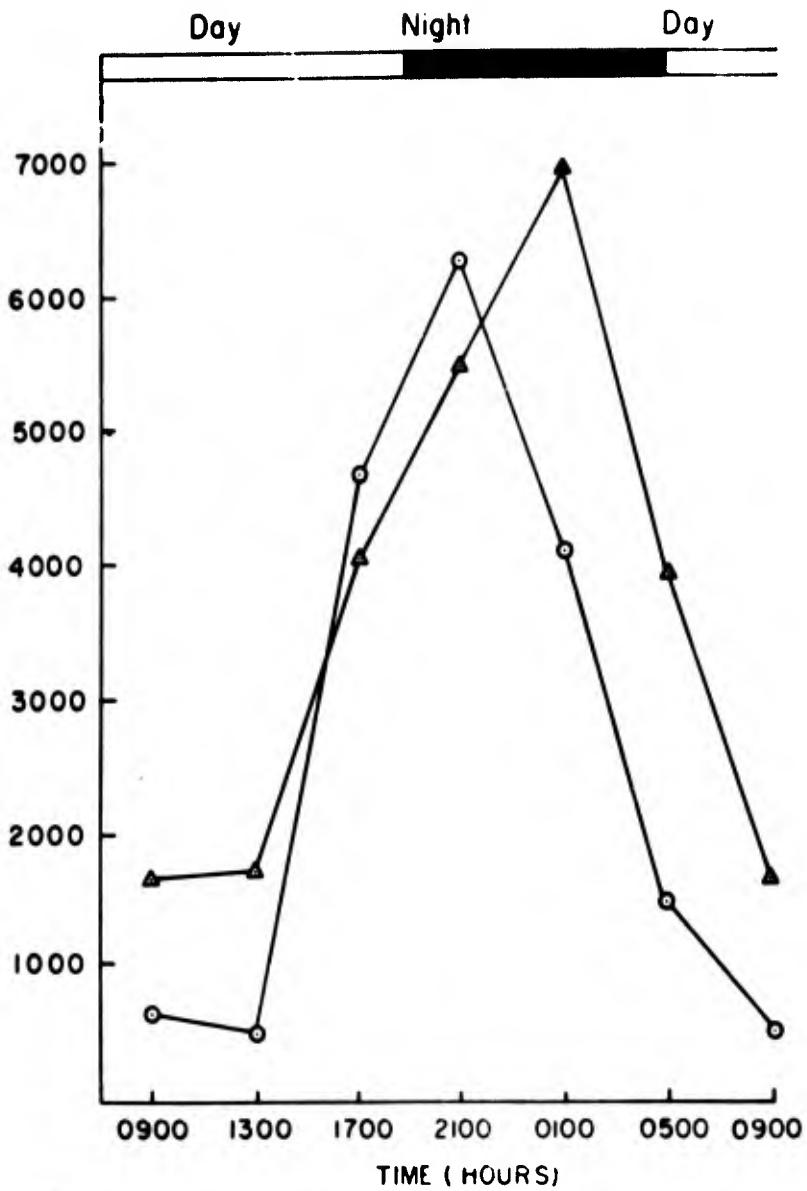


Figure 21. Periodicity of microfilariae of *Macacanema formosana* in *Macaca cyclopis* (Expressed as Microfilariae Per 60. c.mm).

SECTION III

COLLECTION AND IDENTIFICATION OF HEMATOPHAGOUS ARTHROPODS

Filarial Vectors. Most of the work on the transmission of filariae has been concerned with species of medical and veterinary importance. Hawking and Worms (1961) and Lavoipierre (1958a) listed arthropod hosts that included species of *Culex*, *Aedes*, *Mansonia*, and *Anopheles* mosquitoes, various fleas, ticks, and biting flies, e.g., simuliids, tabanids, chrysopids, psychodids, *Stomoxys*, and *Culicoides*.

Manson-Bahr (1959), Raghavan (1956), and Iyengar (1954-60) listed the vectors of *Wuchereria*, Reid *et. al.* (1962) discussed the mosquito vectors of *Brugia malayi* in Malaya. Bibliographies on vectors of onchocerciasis were published by the Pan American Sanitary Bureau (1950,1961). Kartman (1957) discussed the mosquitoes thought to be transmitting *Dirofilaria immitis*. Price (1959) presented 27 parasites in a list of "first observations made of suspected intermediate hosts of filarioid parasites, family Dipetalonematidae." Of particular interest to this paper are the reports of the following parasites with the suspected vector: *Dipetalonema perstans* in *Culicoides austeni*, Sharp (1928); *Mansonella ozzardi* in *Culicoides furens*, Buckley (1933, 1934); *Onchocerca cervicalis* in *Culicoides nubeculosus*, Steward (1932-33); *Onchocerca gibsoni* in *Culicoides pungens* and in *Culicoides oxystoma*, Buckley (1938); and *Onchocerca vulvulus* in *Simulium damnosum*, Blacklock (1926). Steward found that development of *Onchocerca cervicalis* occurred in the midge, *Culicoides nubeculosus*, but not in simuliids, tabanids, and muscoids. Sharp (1928) described the development of *Dipetalonema perstans* in *Culicoides austeni*.

Materials and Methods

Selection of Collecting Areas for Hematophagous Arthropods. When it became evident that all monkeys with filarial infections had been trapped in area 1 between I-lan and Taipei, a concerted effort was made to determine the potential vectors in this area. Eight arthropod sampling areas measuring approximately five by six kilometers were selected. At least one monkey with filarial infection had been trapped within each sampling area. A total of 32 field trips of four to 21 days duration was made to these areas from August 1963 through November 1964. (Fig.22).

Trapping, Collecting, and Preservation of Adult Insects. A live monkey provided the most specific attractant for the intermediate host of the monkey filarial parasite and a trap baited with a monkey was the method of choice in collecting potential vectors of the infection.

The monkeys were carried and housed in light-weight metal cages (Fig. 23.E). The monkey in a cage or on a modified holding rack (Fig. 23.D) was placed inside

Fig. 22. Monkey trapping area near Magan village, **area No. 1, Taiwan**

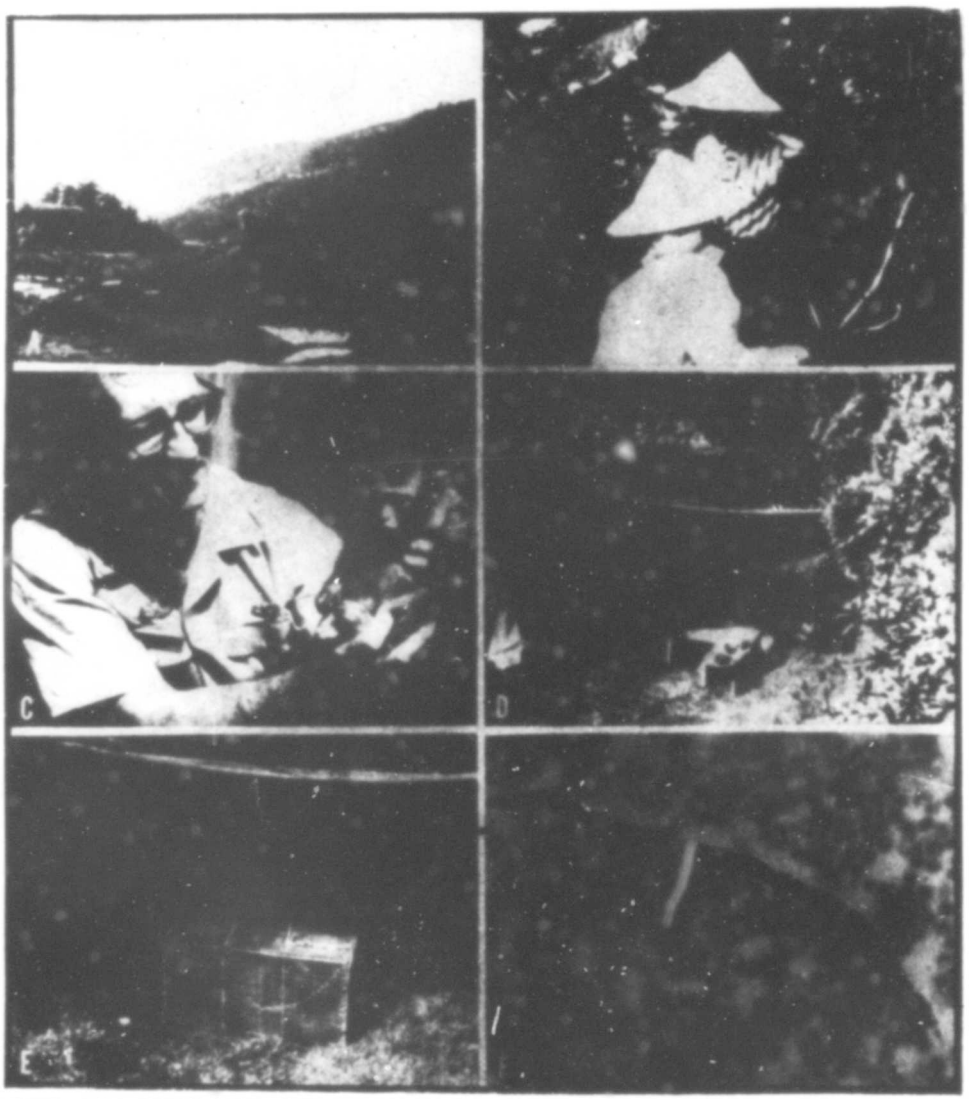


Figure 23. A. Magan village
B. Technicians examining plant leaf axils for *Armigeres omissus* larvae
C. Infant *Macaca cyclopis*
D. Taiwan monkey on restraining board inside modified insect net
E. Monkey in wire carrying cage, placed inside modified insect net
F. Giemsa stained thin smear showing microfilaria described in the peripheral blood of *Macaca cyclopis*

the mosquito net enclosure. The monkey and the net were placed some distance, at least 200 feet, usually upwind, from the campsite to minimize the human effect on the vectors trapped. Different sides of the net enclosure could be raised depending on the climatic conditions. It was discovered early that white netting discouraged the approach of many insects. After the netting was dyed dark olive drab, the variety and number of insects increased dramatically at the same site under the same climatic conditions. The movement of the monkey, confined to the small cage, destroyed many insects that had fed. Therefore. to collect blooded insects, the holding rack was used but the monkey was replaced in the cage after the last collection.

At the time of collection, the team member would enter the net then lower the sides. The insects were detected by flashlight and collected from the monkey and from the sides of the netting with an aspirator. The side of the net would be set again before leaving. Collections were made routinely at 0600, 1000, 1400, 1800, 2000, and 2200 hours. General collections in the area were also made whenever possible. Each team kept a daily diary of events. Data recorded at the monkey site on each collection were: team and station number, date, hour, temperature, weather conditions, technician or team member, and general remarks concerning the animal or the location, The insects at each collection were placed into screw-capped vials containing 70 percent ethanol and labeled.

A New Jersey type light trap was run from sunset to about 2000 hours, on rare occasions, when a generator or batteries were available. The insects collected were anethesized with chloroform and fixed in 70 percent ethanol for later identification.

Tents used as central headquarters had large screened sides that prevented mosquitoes from entering but served as a modified *Culicoides* fly trap. The small *Culicoides* could fly through the screened windows but could not fly back out after a blood meal.

Results

Culicoides Biting in the Field. On 30 November 1964, a filaria positive monkey was taken to the mountain village of Hsiao-i where *Culicoides* had been collected and had been observed biting an uninfected monkey. Between 1700 and 2000 hours, 586 blooded flies were collected, by aspiration, from this monkey. Thirteen flies were dissected immediately following engorgement. Each fly contained from one to 22 motile microfilariae with an average of 5.2 microfilariae per fly. The microfilariae appeared more active in the gut contents of the fly than in the blood of the monkey. The temperature during this specific collection ranged from 13° to 15°C. No flies were collected while biting when the temperature was less than 9.7°C.

Some indication of the number of flies biting, when given the opportunity in the field situation, may be gained from the following example. Each number

represents engorged female *Culicoides amamiensis* caught on a single monkey on the ground and under given conditions. No males flies were caught:

Date	Hours	Temperature (°C)	No. of flies caught
30 November	1700-1800	15	224
30 November	1830-2000	13	100
1 December	1800-1900	13	105
1 December	1900-2000	13	30

Culicoides amamiensis are numerous and bite freely during the evening hours. An individual monkey may receive a large number of bites in a single day. In most cases the flies suck blood from the ventral or under surface of the monkey, especially from the face, abdomen and upper portions of the chest where the hair is sparse. Since midges could be seen and collected with an aspirator more readily on the

Table 5. Summary of mosquitoes collected, females dissected from simian filaria positive area, Taiwan, August 1963 through July 1964.

Species	Females	Males	Total
Aedes albopictus	763	137	900
Ae. elsiae	31	13	44
Ae. hatorii	35	9	44
Ae. japonicus	507	186	693
Anopheles sinensis	447	12	459
An. maculatus	39	0	39
An. minimus	2	0	2
An. ludlowi	3	0	3
Armigeres omissus	3583	126	3709
Ar. subalbatus	323	59	382
Culex annulus	57	0	57
C. bitaeniorhynchus	10	2	12
C. fatigans	22	11	33
C. fuscocephalus	9	0	9
C. mimeticus	10	7	17
C. tritaeniorhychus	919	30	949
Ficalbia luzonensis	2	0	2
Harpagonyis gemurostris	7	0	7
Orthopodomyia anopheloides	15	0	15
Tripteroides aranoibis	6	0	6
T. bambusa	98	0	98
Uranotaenia bimaculata	199	1	200
Total	7087	593	7680

Table 6. List of individual wild insects containing filariae from simian filaria positive area, Taiwan, August 1963 through July 1964.

Species	Date Collected Time	°C Temperature Weather	Filariae Location Measurement	Identity Number Method
Aedes japonicus	12 December 1963	21	thorax	11-1-C-16-A
	0900	light rain no wind	125.4×6.8μ	general collection
Anopheles sinensis	21 August 1960	24	thorax	4-2-H-43-A
	0600	sunny	241.5×9.5μ	general collection
	21 August 1963	22	head	4-2A-69-B
	2010	light rain	—	general collection
Armigeres omissus	23 August 1963	25	mesenteron	4-2A-89-A
	1400	cloudy	—	general collection
	27 August 1963	24.6	head	5-2-I-55-A
	1745	clear	341×18.1μ	woods
Tripteroides bambusa	25 August 1963	27	mesenteron	2-11-38-A
	0800	damp	—	general collection
Culicoides maculatus	20 October 1963	22	mesenteron	7-1-H-15-A
	2000	cloudy	154.8×3.9μ	general collection

Table 7. Species of *Culicoides* collected from simian filaria positive area, Taiwan, 1962-1964

Species	Representative Nos.	Relative Percent
Culicoides		
amamiensis	43,000	82-95
anophelis	2	<0.1
arakawai	732	0.2-1.0
homolomus	8	<0.1
humeralis	1,531	2.5-3.5
maculatus	3,106	4.0-6.9
malayae	3	<0.1
nipponensis	261	0.2-1.0
oxystoma	158	0.2-1.0
tenuipalpis	7	<0.1
palpifer	7	<0.1
Total	48,815	

bare abdomen than when they were concealed in the hair, the hair from the abdomen and chest was removed with clippers.

In the field, there was a great variation in the sensibility of the monkey to the biting of single flies, but every monkey seemed aware of the flies when they were biting in greater numbers. This variation was also noticed by the author and technicians when flies bit them. Some persons felt a slight scratching sensation as the fly began to bite followed by a painful pricking sensation. Finally, an intense irritation was apparent just as the fly was ready to move away. Swelling and edema would sometimes persist for two weeks following the bites. On others the fly caused no sensation even when attention was directed to detecting bites.

Dissections: Over 7,000 mosquitoes of 22 species were collected in the simian filaria area from filaria negative monkeys, identified, and sexed; the females were then dissected (Table 5). The proboscis, the thorax, and abdomen, in that order, were separated and each placed in separate drops of saline or 0.02 percent methylene blue in two percent saponin. The tissues were dissected, examined microscopically for developing filarial larvae. Filarial larvae of unknown origin were found in six of the mosquitoes collected from the area in which infected monkeys were collected.

A microfilaria or larva was found in the mid-gut of a single ***Culicoides maculatus*** from the general collection (Table 6).

Eleven species of ***Culicoides*** were identified from light trap collections from known filarial positive monkey areas. The relative percentages of the species are given (Table 7). Over 1000 ***Culicoides*** flies were dissected and examined for filarial larvae. Although no microfilariae were found, this does not imply that ***Culicoides*** could not serve as a suitable vector of ***Macacanema formosana***. ***Culicoides*** have a relatively short flight range, generally less than 1-2 miles, but the flies were numerous enough to act as vectors of simian filaria if they were biologically suitable.

SECTION IV

ARTHROPODS AND LABORATORY TRANSMISSION EXPERIMENTS

Introduction The purpose of this section is to describe the attempts, methods, and results of experimental infections of various arthropods. Other than relative numbers (Tables 5, 6 and 7) there was little early evidence from the large number of available monkey biting arthropods that indicated to what group of insects the vector would most likely belong. Experimental work with various species of insects often has been based mainly on circumstantial evidence. In the paper by Hawking and Worms (1961), "A list of the filarial worms known to develop in arthropods," served as the initial reference point.

The first stage of the transmission of a filarioid worm is the passage of the microfilariae from the vertebrate host into a blood sucking arthropod (Hawking and Worms, 1961). Passage is influenced by the distribution of the microfilariae in the subcutaneous tissues or capillaries of the vertebrate host, the timing of the microfilariae peak in the blood and the maximum biting activity of the vector, by vector factors, such as the height above ground in the forest canopy at which the vector and the vertebrate host occur, how readily the microfilariae pass into certain hematophagous arthropods (Chardome and Peel, 1949), and the minimum density that would still infect a vector (Rosen, 1955). Factors inherent in the vertebrate host have been described by Bertram *et al.* (1949, 1950, 1957), Gordon (1955), Anderson (1956), and by Hawking and Worms (1961). For successful transmission by an insect, *M. formosana* microfilariae would have to be present in relatively small quantities of blood. The marked nocturnal microfilariae periodicity in the peripheral blood of the monkey increased the probability that a nocturnal feeding insect was the vector, but it was decided to investigate the possibility of other arthropods as well.

Lavoipierre (1958a, b) reported that among hematophagus insects, complete development of filarial worms to the infective forms has been shown to take place in mosquitoes (culicids), buffalo-flies (simuliids), fleas, psychodids, some ticks and mites, as well as uncertain development in a grasshopper and in biting lice. Lavoipierre expressed his surprise that despite the many dissections, the tsetse flies and blood sucking bugs have never been shown to act as vectors of filarial nematodes. Yao *et al.* (1938) indicated that microfilariae of *Wuchereria bancrofti* could develop to the post-sausage stage in the sandfly, *Phlebotomus sergenti* var. *mongolensis*. The development of the various filaria has been described by Lavoipierre (1958b) as taking place with a high degree of specificity in the arthropod, i.e., *W. bancrofti* in the muscles, *Litomoscides carinii* in the fat body, *D. immitis*,

D. repens, and *D. tenuis* in the Malpighian tubules, but he doubts development takes place in the hemocoel proper of the intermediate host.

On these grounds, it seemed reasonable to look for the vector of *Macacanema formosana* in a group of insects related to the simuliids and ceratopogonids. The search would therefor be confined mainly to the families of biting insects included in the Nematocera, i.e., Ceratopogonidae (*Culicoides*); Psychodidae (*Phlebotomus*); Simuliidae (*Simulium*); and the Culicidae (various mosquitoes). Some of the arthropod larvae and adults were collected in the simian filaria positive area of Taiwan, others were from various laboratory colonies in Taiwan and from the United States (Table 8). The biting flies of the genus *Culicoides* have often been overlooked because of their relative small size but they have been incriminated as vectors in the transmission of bluetongue virus disease in sheep and cattle (Bowne and Jones, (1966), of the American frog filaria *Icosiella quadrituberculata* (Pechuman and Wirth, 1961), and of *Hepatocystis kochi* from *Cercopithecus aethiops* in the midge *Culicoides adersi* (Garnham *et al.*, 1961).

Arnaud and Wirth (1964) with the addition of 245 new names, brought the names proposed within the genus *Culicoides* to a grand total of 916. They noted that synonymy and generic transfers were not considered and estimated there remained some 800 valid *Culicoides* species. Wirth and Hubert (1961) listed and gave a key to the species of Taiwan *Culicoides*. Eighteen species are recorded, most of them are common and widespread throughout the orient. Sun (1961) also included *Culicoides formosae*, *C. indecora*, and *C. sumatra*. These last 2 papers bring to date the work of Tokunaga (1937) and should be used in conjunction with Arnaud's (1959), and Wirth and Hubert's (1959, 1961) papers.

Materials and Methods

Aedes aegypti, *Aedes albopictus*, *Anopheles quadrimaculatus*, *Culex fatigans*, *Culex tritaeniorhyncus*, *Rhodnius prolixus*, *Ornithodoros tartakovskyi*, *Culicoides guttipennis*, and *Culicoides variipennis* were available in established colonies elsewhere; for the standard materials and methods, the reader is referred to the literature or contributor acknowledged in each section that follows.

Insectaries Insectaries were established at NAMRU-2, Taipai, Taiwan and at NMRI, Bethesda, Maryland, primarily for the purpose of rearing and maintaining arthropods which were not maintained in colony elsewhere and for arthropods collected as larvae, pupae, and adults from the field.

The insectary at NAMRU-2 was a 6 by 12 ft. room in which the temperature was maintained between 16° and 30°C. by manually regulating the 150 watt incandescent bulb mounted inside each of the three 1 ft. sq. wooden boxes. Relative humidity in the room was increased by allowing water to move by capillary action down towels or sheeting suspended from 5 gal. pans containing water to catch pans

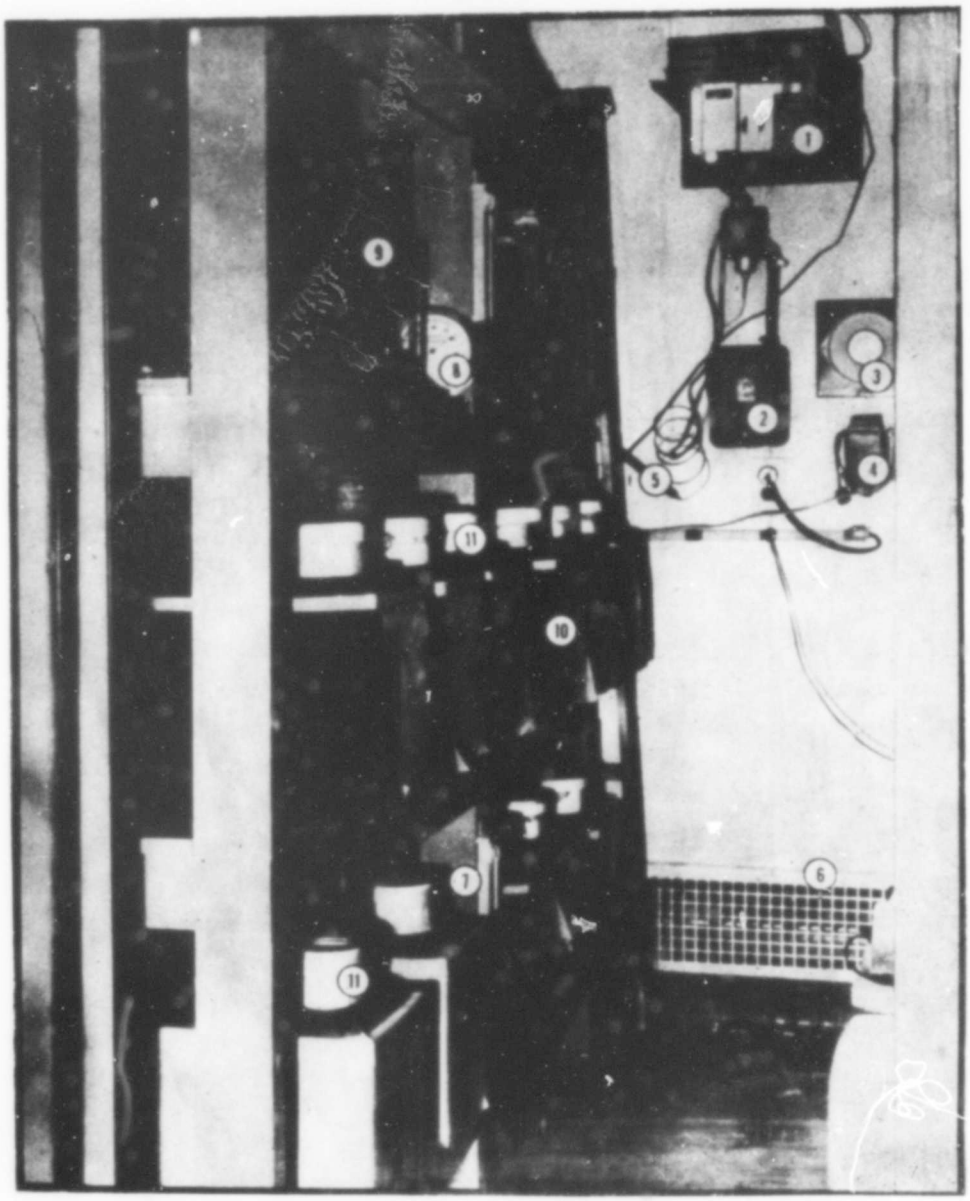


Figure 24. *Culicoides* insectary, NMRI, Bethesda, Maryland
(1) humidifier; (2) thermostat relay; (3) fan;
(4) fan rheostat; (5) thermorod; (6) heater;
(7) minimum-maximum thermometer;
(8) hygrometer; (9) hygrothermometer recorder;
(10) painted five gallon aquarium;
(12) 1/2 pint carton for adult fly retrieval.

on the floor. The water was replenished daily. In addition, the individual screened cages were covered with moist towelling or untreated canvas. An electric fan was used to circulate the air. An approximate 12:12 light:dark cycle was provided by 4 daylight, 40 watt, fluorescent lamps.

The *Culicoides* insectary at NMRI was a converted walk-in incubator box (Fig. 24). A constant temperature ,26.5 ± 2°C,, was maintained by a thermo-statically-controlled baseboard heater. A relative humidity of 80 percent ± 5 percent was maintained by a centrifugal force humidifier with a constant water supply regulated by a float valve. Adult flies were maintained under the above conditions in semi-darkness (less than 1 ft. candle) at all times except while being attended. A wall fan circulated and brought in fresh air.

Larval rearing was conducted in painted 5 gallon aquaria in complete darkness except for the light that diffused through the emergence cups (Fig. 24). The adult holding cages, methods of egg collecting, larval media, adult diets, the generalized life cycle, and the procedures for laboratory colonization and mass-production of *Culicoides guttipennis* are described in detail by Hair and Turner (1966).

Preparation of the Monkey as the Donor for Arthropod Feeding. For experimental feeding in the laboratory, the monkey was placed on its back on a restraining board of the type used in the field collections (Fig. 23.25). Two stocks held the head and legs in position and the arms were secured by straps to the base of the restraining board, allowing minimum of movement. The ventral surface of the abdomen and chest was closely shaved, washed first with an antiseptic detergent and water and then thoroughly rinsed with clean water to remove all trace of the detergent.

No general anesthetics were necessary during most feedings. However, when large numbers of *Culicoides*, *Ornithodoros*, or *Rhodnius* were fed, the monkey was first anesthetized with veterinary sodium nembutal. A tranquilizer, promozine hydrochloride, 2 mg. per lb. of body weight, was used on occasion.

The monkey was inverted on the restraining board when feeding negative geotropic *Culicoides* (Fig. 25). The flies in a carton covered with nylon netting were enticed by the spot of light directed on the abdomen of the monkey by the microscope lamp and would move to that area to feed. The top of the carton was fitted through a hole cut in the wooden table. The monkey was tied securely to the table to prevent movement that would disturb the flies. It was not necessary to invert the monkey when exposing most mosquitoes, *Ornithodorus* or *Rhodnius* The abdomen and chest were washed again with detergent upon completion of vector feeding to prevent secondary infection and to ease subsequent pruritis.

Insect Membrane Feeder A membrane feeder was used when feeding *Aedes aegypti* in which the blood firmly coagulated. Heparinized blood drawn from an infected monkey at the peak of microfilariae periodicity was placed into

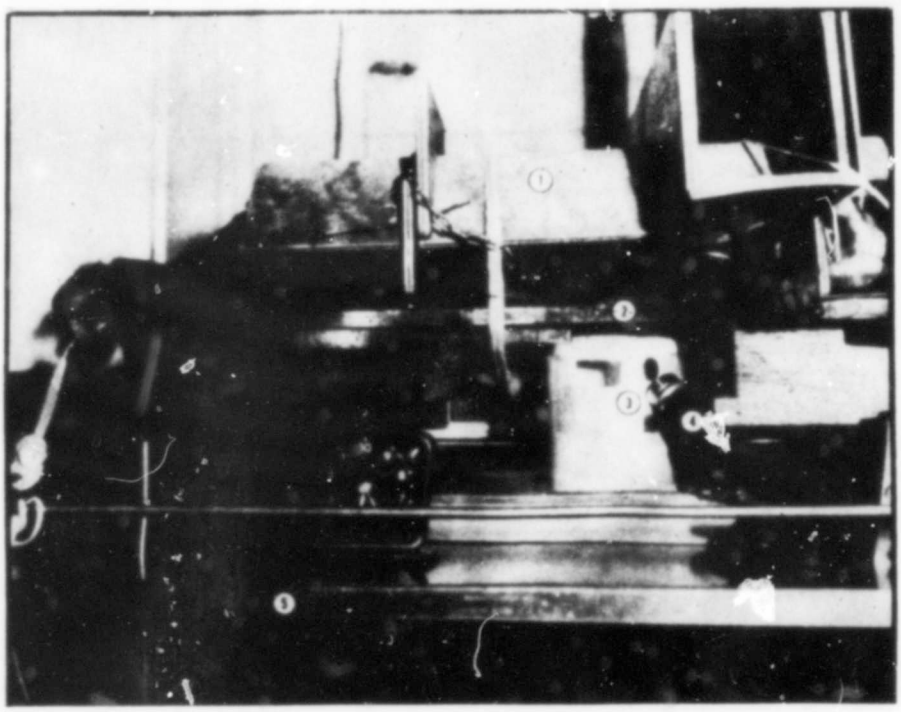


Figure 25. Insect feeder set up utilizing filarial infected monkey (See also Figs. 23,29).

(1) Monkey restraining board, inverted
(2) wooden table with 18 c.mm. hole for insertion of
(3) 1/2 gal. cardboard carton containing insects to be fed, note plastic covered holes
(4) microscope lamp
(5) portable table

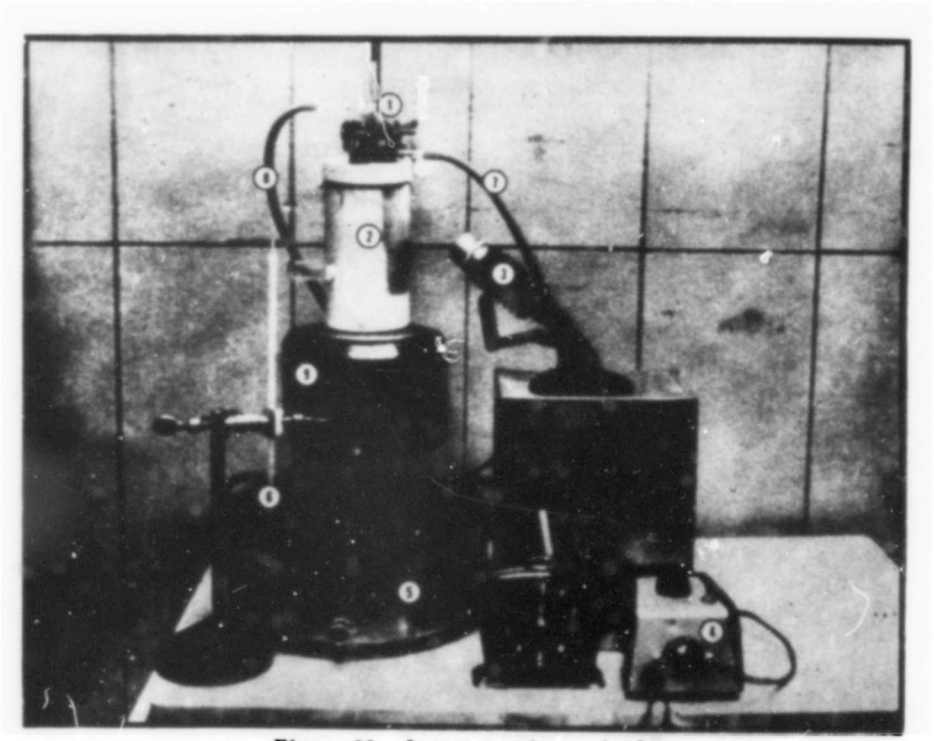


Figure 26. Insect membrane feeder

(1) Glass water jacket surrounding membrane covered tube containing heparinized monkey blood
(2) pint carton containing insects
(3) microscope lamp
(4) light rheostat
(5) water bath, heater-regulator
(6) thermometer
(7) water input tube from water bath to water jacket
(8) water output tube
(9) centrifugal water pump

a membrane-covered tube surrounded by a water jacket (Fig. 26). A spot of light from the lamp was directed through a hole in the carton and onto the membrane. Cow intestinal membrane and scraped skin from a young chicken were used as membrane covers. Both membranes were soaked in warm physiological saline for 5 to 10 minutes prior to feeding exposure.

Preparation of the Vector The vector as well as the donor monkey must be properly prepared if the feeding is to be a success. Mosquitoes were generally held on glucose and water for at least 3 days after emergence. Beginning on the 3d day and 12 to 24 hours prior to the blood meal mosquitoes were given only water and maintained in dark or semidark containers. The darkness preconditioning was not necessary with the *Aedes*. No more than 100 mosquitoes were placed in a quart container to prevent over-crowding.

The *Culicoides* were able to feed almost immediately after emergence. However, as a standard, 3-day-old flies were used and they were maintained on sugar water for the first 2 days. Water only was given the day of the blood meal. Feeding took place in semi-darkness in which the only source of light was from the microscope lamp.

The *Rhodnius prolixus* and *Ornithodoros tartakovskyi* were blood starved for 3 to 4 weeks prior to monkey exposure. The *Stomoxys* and *Chrysops* collected from cattle were blood starved for 48 to 72 hours prior to exposure; water only was available the day of the blood meal.

Experimental Vectors

A summary of the experimental arthropods exposed and fed on Taiwan monkeys with natural infections of *Macacanema formosana*, from 1962 to 1967, is given (Table 8). The 22 species of arthropods exposed can be divided into 6 major groups: The Culicidae (15 strains or species of mosquitoes); the Tabanidae (*Chrysops*) and the Muscidae (*Stomoxys*); Ceratopogonidae (3 species of *Culicoides*); Simuliidae (*Simulium rugglesi*); Argasidae (*Ornithodoros tartakovskyi*); and Reduviidae (*Rhodnius prolixus*). The arthropod source and the relative percentages of those that fed are presented.

Attempted Infection of Mosquitoes with *Macacanema formosana* The *Aedes aegypti* and *Anopheles quadrimaculatus* were obtained from Dr. Levon A. Terzian and Mr. Nathan Stahler of NMRI. The mosquitoes were reared and maintained at a constant temperature of 26.5°C. and a relative humidity of 70 to 75 percent (Stahler, 1959; Terzian and Stahler, 1949). *Aedes albopictus*. *Culex fatigans*, and *Culex tritaeniorhynchus* were provided by Dr. Herbert S. Hurlbut. All mosquitoes, three to 10 days old. were starved for 24 hours prior to a 1-hour bloodmeal exposure. A quart container covered with bobbinet containing less than 100 female mosquitoes was inverted over the shaved abdomen of the infected monkey.

Table 8. Summary of arthropods experimentally fed on Taiwan monkeys with natural infections of *Macacanema formosana*, (1962-1967).

Species	Source	Exposed	Fed	Latest day parasites noted
Aedes aegypti	NMRI colony Bethesda, Maryland	100	90	3
Ae. aegypti	Penghu Islands Pescadores	571	427	5
Ae. albopictus	NAMRU-2 colony Taipei	150	80	5
Ae. hatorii	Taipei Hsien	57	4	4
Ae. japonicus	Taipei Hsien	142	3	4
Ae. togoi	Ali-Lo, Taipei Hsien	336	102	4
Ae. triseriatus	College Park, Maryland	35	15	3
Anopheles quadrimaculatus	NMRI colony Bethesda, Maryland	95	75	4
An. minimus	Pin-ling, Taipei Hsien	—	103	4
An. sinensis	Taipei Hsien	474	198	4
Armigeres omissus	Wu-Lai, Taipei Hsien	—	484	4
Ar. subalbatus	I-Lan- Taipei Hsien	920	260	4
Culex fatigans	NAMRU-2 colony Taipei	—	204	4
C. tritaeniorhynchus	NAMRU-2 colony Taipei	—	360	4
Mansonia uniformis	Mu Chu, Taipei Hsien	—	85	4
Stomoxys sp.	Taipei	7	6	3
Chrysops sp.	Taipei	12	8	3
Rhodnius prolixus	NMRI colony Bethesda, Maryland	43	23	25
Ornithodoros tartakovskyi	NIH colony Bethesda, Maryland	200	153	28
Culicoides amamiensis	Wu-Lai, Taipei Hsien	224	100	31
C. guttipennis	VPI colony Blacksburg, Virginia	373	13	9
C. varipennis	USDA colony Denver, Colorado	227	131	16
Simulium rugglesi	USDI lab., Laurel, Maryland	20	0	—

The non-anesthetized monkey was immobilized on a restraining board. The microfilariae density in the monkey blood was determined before and after the blood meal. Mosquitoes that took a blood meal were separated and maintained at 26° to 30°C. Each cage was covered with water soaked cotton. Fresh 10 percent dextrose was provided daily.

Representative mosquitoes were dissected immediately to determine whether or not the mosquitoes had ingested microfilariae. Mosquitoes were anesthetized with chloroform, then the wings and legs, head, thorax, and abdomen separated and placed in different drops of physiological saline on a glass slide and examined. Dissection of the mosquitoes and examination for microfilariae was first made under a stereoscopic dissecting scope and then examined under a compound scope at 100X and 440X. Mosquitoes were dissected from 12 hours to 30 days post blood meal to trace the development of the filarial larvae. Depending on the species of mosquito it appears that the microfilariae pass out with the blood dejecta in 48 to 72 hours, e.g., ***Culex fatigans*** and ***Culex tritaeniorhynchus*** (Table 9). No microfilariae were noted later than day 5 (Table 8). No development of the microfilariae was observed.

Development of *Macacanema formosana* Larvae in *Culicoides amamiensis*

The microfilariae taken up in the monkey's blood by the midge passes to the abdomen with the blood. The microfilariae may be found in the midgut of the fly on the edges of the gelatinous blood mass up to 72 hours after the blood meal is ingested. The development of the larvae is shown in Fig. 27.

A slight increase in the average width of the larvae is noticeable at 48 hours and by 6 days the larvae have shortened and thickened considerably (Fig. 27A). The "sausage stage" in the thoracic muscle measured 97.2×8.6μ. There was little larvae movement and no evidence of progressive movement. Development and growth sometimes takes place at an unequal rate. The anterior extremity is somewhat bluntly rounded and the posterior extremity tapers to a spike-like tail that remains somewhat motile. The larvae appear to be uniformly filled by granules of varying sizes which stain deeply with Giemsa or hematoxlin; the larger granules resemble nuclei and are arranged in fairly regular lines. Growth continues to take place and a width of up to 12μ. may be obtained in this stage.

At 15 days the larvae may measure 126×7.2μ. (Fig. 27B) and still have the sharp pointed tail appendage that was so noticeable in the short and stout "sausage stage". Ecdysis takes place and lateral movements are more noticeable in the larvae. The internal structure now appears more definite and the developing digestive tract can seen. Later the length of the larvae increases. At 16 days, 3d stage larvae measuring 437×14.6μ. are noted in the head of a dissected *C. amamiensis*. However, a motile 3d stage larvae that measured only 340.2×10.2μ. was noted in the hemocoel of another fly at 22 days. After the 16th day the infective forms

Table 9. Summary of microfilaria in *Culex fatigans* and *Culex tritaeniorhynchus* at various intervals post blood meal (Kim and Bergner, 1964).

Time dissected	No. of dissected mosq.		No. of mf. found		Mean no. per. mosq.	
	C. fat.	C. tri.	C. fat.	C. tri.	C. fat.	C. tri.
Immediately	16	15	371	140	23.2	9.3
12 hours	15	15	224	75	14.9	5
24 hours	15	10	77	36	5.1	3.6
36 hours	12	10	15	1	1.3	0.1
48 hours	9	15	6	0	0.7	—
3 days	8	11	0	0	—	—
4 days	8	15	0	0	—	—
5 days	8	9	0	0	—	—
6 days	8	10	0	0	—	—
7 days	8	9	0	0	—	—
8 days	8	16	0	0	—	—
9 days	9	2	0	0	—	—
10 days	15	15	0	0	—	—
11 days	15	13	0	0	—	—
12 days	15	19	0	0	—	—
13 days	15	19	0	0	—	—
14 days	15	19	0	0	—	—
15 days	—	13	—	0	—	—
16 days	—	18	—	0	—	—
17 days	—	20	—	0	—	—
18 days	—	21	—	0	—	—
19 days	—	22	—	0	—	—
20 days	—	20	—	0	—	—
21 days	—	10	—	0	—	—

may be found in the head. On the 22d day, motile larvae were seen to emerge from the base of the proboscis in an intact *Culicoides amamiensis* when the head of the fly was placed in saline. The head forms ranged from 324×14.6μ. to 447×14.6μ.

Development of *Macacanema formosana* Larvae in *Culicoides variipennis*. The development of *Macacanema* larvae in *Culicoides variipennis* (Fig. 28) at the U.S. Department of Agriculture Laboratories, Agricultural Research Service, Denver, Colorado, was made possible through the cooperation of Dr. Robert Henry Jones and Dr. John G. Bowne. All operations with flies involving the monkey were performed inside a plexiglass isolation chamber (Jones, 1966) located in an insect proofed room. Female flies 24 to 72 hours old were allowed to feed on an infected monkey or through a membrane containing heparinized blood drawn

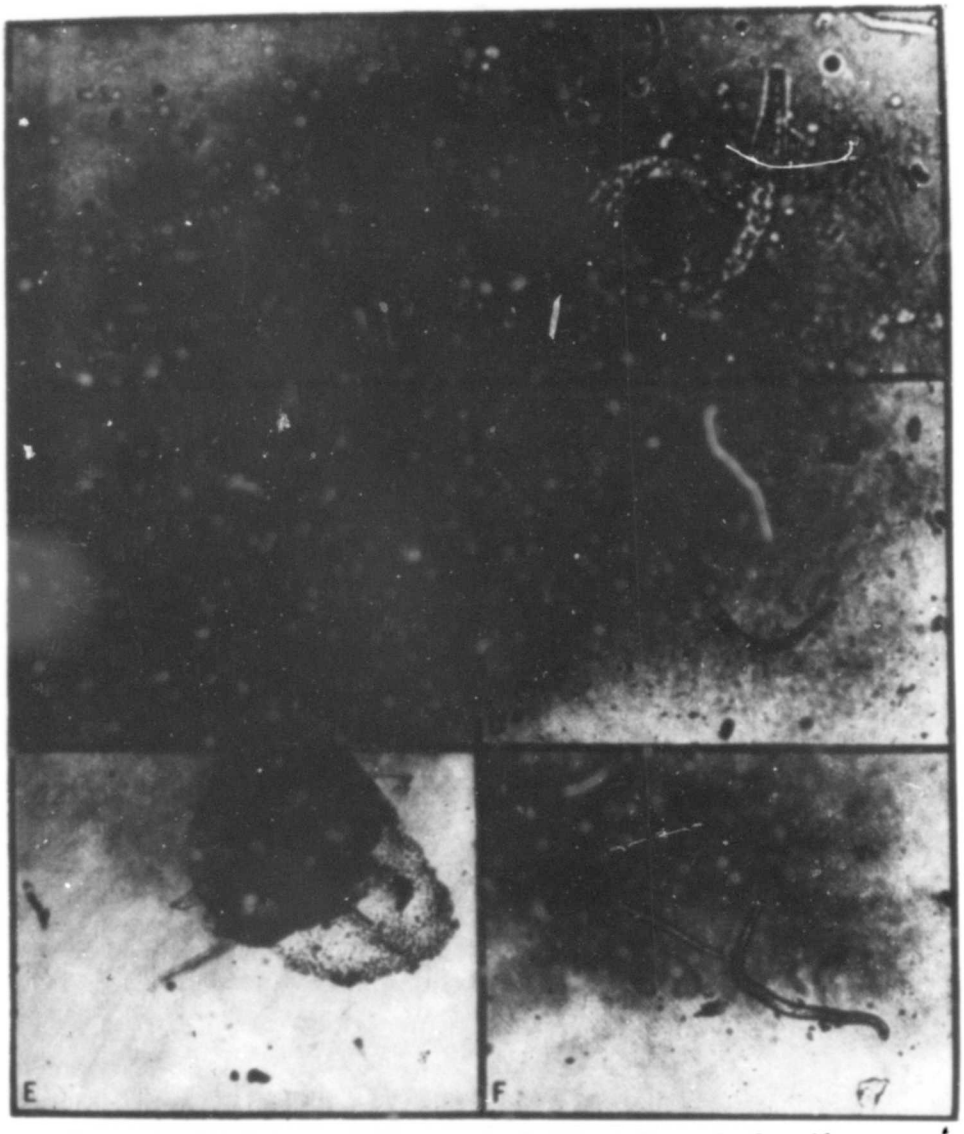


Figure 27. Development of *Macacanema formosana* larvae in the midge, *Culicoides amamiensis* from area No. 1, Taiwan, reported as days post infective blood meal from *Macaca cyclopis*

A. "Sausage stage" larva in the thorax at 6 days, 97.2×8.6μ.

B. Second stage larva in the thorax at 15 1/2 days, 126×7.2μ.

C. Motile 3d stage larva in the hemocoel at 22 days, 340.2×10.2μ.

D. Motile 3d stage larva in the head at 16 days, 437×14.6μ.

E. Infective larva in the head of *Culicoides amamiensis* at 21 days, 447×14.6μ.

F. Motile larvae emerged from proboscis of *Culicoides amamiensis* when head was placed in warm saline at 22 days, 349×14.6μ. and 324×14.6μ.

Figure 28. Development of ***Macacanema formosana*** larvae in the midge, ***Culicoides variipennis***, experimental, reported as days post infective blood meal from ***Macaca cyclopis***.

A. Larva in the thorax at 6 days, dissected from flight muscle, 120×8μ. (low power).

B. Larva in the thorax at 6 days, 118×6.6μ.

C. Fragment of larva in the thorax 10 days, ≦ 125×5μ.

D. Larva dissected from thoracic muscle at 10 days, 204×5μ.

E. Larva in the thorax at 12 days, 180×5μ.

F. Larva in the thorax at 16 days, 382×9.5μ.

between 2100 and 0100 hours from an infected monkey. Each fly was observed under the microscope to insure that it had taken the minimum amount of blood to distinctly swell the abdomen.

Inoculated flies were maintained in the isolation chamber at 24° ± 2°C. The flies were kept in half-pint cardboard cages and fed an alternating diet of water and 5 percent sugar solution (Jones and Foster,1966). Sugar solution was not provided the day before flies were used in an experiment. The only blood meal was the experimental feeding of infective blood. The flies were anesthetized with carbon dioxide gas to facilitate handling.

Development in *Culicoides variipennis* follows a pattern similar to the growth in *Culicoides amamiensis*. Larvae noted in the thorax at 6 days measured 118-120 ×6.6-8μ. Again measurements varied with the development and growth of the larvae at an unequal rate. Larvae dissected from thoracic muscle at 10 days measured 204 ×5μ. The internal structure was more definite and the digestive tract could be seen in some of the specimens examined. A larva in the thorax, at 16 days, measured 362×9.5μ. was motile and serpentine. It is assumed that these forms would have proceeded to the infective stage in a period of time similar to those larvae in *Culicoides amamiensis*.

Notes on the Development of *Macacanema formosana* in *Culicoides guttipennis*. *Culidoides guttipennis* larvae were obtained through the courtesy of Dr. Donald Messersmith, Dr. Jakie Hair, and Dr. E.C. Turner, from the insect colony at Virginia Polytechnic Institute, Blacksburg, Virginia. The larvae were reared and the adult fly colonized. Only 13 of 373 female flies took a blood meal from a *Macacanema formosana* infected monkey. Considerable difficulty was experienced in enticing the flies to take a blood meal on the monkey. However, flies dissected immediately after a blood meal demonstrated motile microfilariae' "Sausage stage" forms were seen in flies dissected at 5 and 9 days respectively. It is concluded that *Culicoides guttipennis*, generally an avian feeder, will feed on the Taiwan monkey with difficulty; the larvae of *M. formosana* did develop to the "sausage stage" and there is the probability that the larvae could develop further with longer survival of the fly.

Attempted Development of *Macacanema formosana* in *Rhodnius prolixus* Nymphs and adults of the triatomid bug, *Rhodnius prolixus*, were obtained through the cooperation of CDR. Alan C. Pipkin, Sr., MSC USN. The eggs, nymphs, and adults were reared in the triatomid insectary, NMRI, following, in general, the methods of Gomez-Nunez (1963), Harrington (1960), Ryckman (1952), and Wood (1963). Temperature was maintained at 26° to 28°C.; relative humidity ranged from 83 to 86 percent. Uninfected bugs were maintained by feeding them every 3 to 4 weeks on clean guinea pigs.

Both adult and nymph stages of *Rhodnius prolixus* were permitted to feed for

one-half hour on a filaria positive monkey beginning at 2130 hours. Representative bugs were dissected immediately following the blood meal. Great numbers of microfilariae were noted in the blood meal of all bugs that fed (Table 8). The bugs were dissected on the 25th day after the infective blood meal. Microfilariae were still present in the gut, all were dead or non-motile, and no development but some disintegration had taken place. No larvae were found in the tissues outside the gut.

Attempted Development of *Macacanema formosana* in *Ornithodoros tartakovskyi* Nymphs and adults of the argasid tick, *Ornithodoros tartakovskyi*, were obtained through the cooperation of Dr. Guillermo Pacheco. The ticks were reared in the insectary, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, following, in general, the methods of Worms *et al.* (1961) and Baltazard *et al.* (1953). Temperature was maintained at 25° to 28°C; relative humidity at 80 percent. Uninfected ticks were maintained by feeding them every 3 to 4 weeks on new born rats.

The filaria infected monkey was anesthetized with sodium pentobarbital. Two hundred adult and nymph *Ornithodoros tartakovskyi* were permitted to feed on the shaved abdomen of the monkey for one-half hour beginning at 2230 hours. Coxal fluid was secreted during engorgement. Individual tick bites could be recognized and there was a circumscribed subdermal effusion of the blood up to 11 mm. in diameter surrounding the bite (Fig. 29). The wound continued to "ooze" for several hours. This type of reaction by the monkey was not seen in any of the other insect feeding experiments.

Representative ticks of both stages were dissected immediately following the blood meal. Of the 200 ticks exposed, 153 took a satisfactory blood meal. Ticks were dissected at various intervals throughout the 29-day holding period. Microfilariae were noted in all ticks that fed (Table 8). Motile microfilariae were occasionally seen up to 7 days after the blood meal. The microfilaria seen in ticks dissected on the 29th day were dead or non-motile, no discernible development was noted and some disintegration had taken place.

Attempted Infection of *Chrysops* and *Stomoxys* Few *Stomoxys* sp. and *Chrysops* sp. were collected from a cattle shed prior to engorgement and blood starved for 48 to 72 hours with only water available the day prior to exposure on the infected monkey. Most of the flies took a blood meal (Table 8). Many motile microfilariae were seen in the contents of the blood meal of those flies dissected immediately. No microfilariae were noted beyond the 3d day.

Notes on *Simulium ruggleai* Adult female flies received from Dr. Barry I. Tarshis, U.S. Department of the Interior, Laurel, Maryland, were blood starved for 48 hours with only water available the day prior to the initial exposure on the infected monkey. The flies refused to take a blood meal on 3 consecutive days;

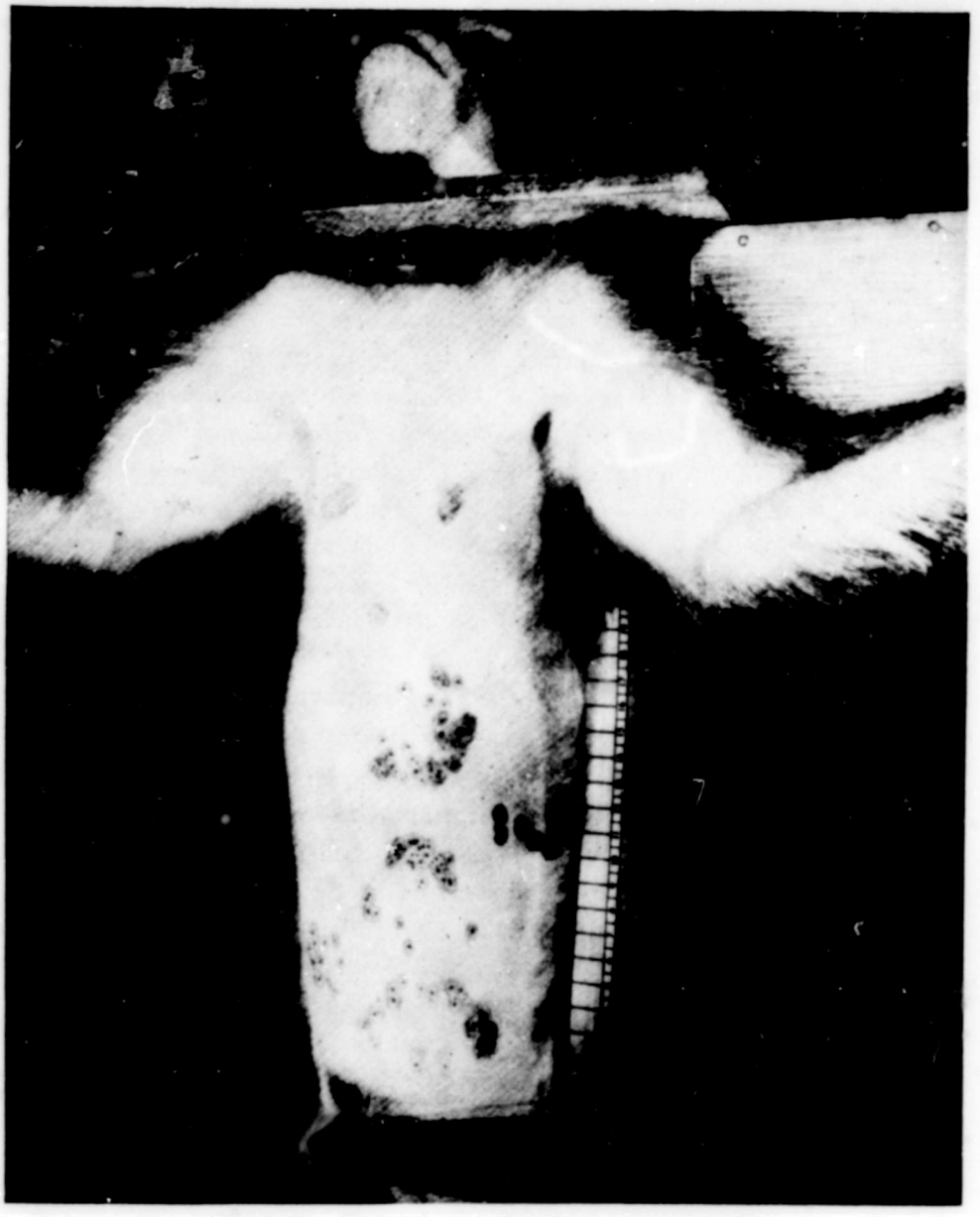


Figure 29. Tick bites by *Ornithodoros tartakovskyi*

however, they took a glucose meal when offered on the 6th day. None of the flies survived beyond the 9th day.

Discussion and Conclusions Conclusions drawn from the early survey work of the writer suggested that mosquitoes, simuliids, or ceratopogonids were the most likely vectors of *Macacanema formosana* in Taiwan. Mosquitoes have been excluded on experimental and circumstantial evidence. Attempts to feed *Simulium* were unsuccessful. Subsequent investigations with *Culicoides* have provided the most conclusive evidence that they are the probable transmitters of simian filaria in Taiwan.

The development of the larvae of *Macacanema formosana* in the midge, *Culicoides amamiensis* and *Culicoides variipennis*, is similar to the development of other dipetalonematids in their intermediate hosts (Figs. 27, 28). The changes in the dimensions of the developing larvae of *Macacanema formosana* are similar to those given by Anderson (1956) for *Ornithofilaria fallisensis*, by Feng (1936) for *Wuchereria bancrofti*, and by Kartman (1953 a, b) for *Dirofilaria immitis*. Chandler *et al.* (1941) gave an excellent series of illustrations showing the development of *W. bancrofti*. The change in the dimensions of some of the other species have not been followed closely, but from the illustrations and descriptions of the larval stages in the literature it can be inferred that most develop similarly. The peculiarity of the growth of the larvae of these species is the formation of a "sausage stage," a form which has thickened and contracted until it is shorter and several times thicker than the original microfilariae in the definitive host.

The humidity conditions under which the arthropods were held require some comment. Basu and Rao (1939) found that in mosquito-borne filariasis, the relative humidity affected the infection rate in mosquitoes. Relative humidity of over 70 percent was favorable whereas development of the worms restricted at 60 percent relative humidity. However, Bertram *et al.* (1950) found little or no effect on the efficiency of the blood sucking mite, *Liponyssus bacoti*, to transmit *Litomosoides carinii* to the cotton rat at either 50 percent or 80 percent relative humidity. As with Sharp's (1928) work with *Culicoides austeni*, it is probable that *Culicoides amamiensis* requires 2 blood meals to complete ovulation. A high relative humidity was essential for the well-being of *Culicoides amamiensis* and for the development of the ova.

Jamnback (1961) demonstrated that wild caught *Culicoides obsoletus* survived up to 51 days in captivity and that flies without a blood meal but given a sugar solution survived about as long as flies with a blood meal. Flies that were given sugar solution lived about 5 times longer than flies given only water. Initial difficulty encountered in keeping *Culicoides amamiensis* alive was overcome by blood feeding at 2-to-5-day intervals, increasing the relative humidity, and maintaining the flies in semi-darkness. The flies appeared to feed more readily on warm

moist days and engorement on a restrained monkey usually took only 3 to 8 minutes. While the fly was feeding it would sometimes excrete a small drop of glistening clear fluid from its posterior end.

The high relative humidity did not appear to be essential when working with *Culicoides variipennis;* an average relative humidity of 18 percent was maintained in the Denver laboratory. However, the possible effects of a higher relative humidity on the longevity of the fly, the maintenance, and the development of the parasite in *Culicoides variipennis* should be investigated further.

According to Anderson (1956), the transmission of *Onchocerca volvulus* to humans in Central America is believed to depend not only on the presence of the proper fly vectors and infected persons but also upon their respective population densities. Buckley (1938) demonstrated that although the individual insect was inefficient, the genus *Culicoides* taken as a whole is an efficient vector of *Onchocerca gibsoni* in cattle. However, Bertram *et al.* (1950) had shown that the frequency of the different numbers of microfilariae ingested by the mite of the same batch is a skew distribution; the majority of mites took up smaller numbers of microfilariae than occur in an equivalent quantity of blood in the cotton rat's peripheral circulation. As the microfilariae density decreases, the likelihood of any mite failing to ingest at least a few microfilariae becomes progressively greater. This would be true of any vector, including that of *Macacanema formosana.*

Another factor that should be considered is that some of the circulating microfilariae may be non-infective and vectors acquiring only the non-infective forms would fail to develop infective larvae. There may also be variable resistances in the vector.

The exposed experimental vectors fall into 5 groups based on the fate of the microfilariae from the peripheral blood of the Taiwan monkey: (1) *Simulium rugglesi* would not feed or take a blood meal from the Taiwan monkey. (2) *Pedicinus eurygaster* taken from filaria positive monkey at capture contained plasma or body fluids from the monkey, but no microfilariae were noted in these lice. (3) *Stomoxys, Chrysops,* and 5 genera, 14 species of Culicidae took a blood meal containing active, motile microfilariae. However, the microfilariae passed from the mosquito or fly in 48 to 120 hours, depending on the species, with the blood dejecta and with no development of the microfilariae. The Culicidae have failed to justify any belief that a mosquito might serve as a vector of *Macacanema formosana.* (4) The argasid tick, *Ornithodoros tartakovskyi* and the reduviid, *Rhodnius prolixus,* took a blood meal containing motile microfilariae. In contrast to the culicids, the tick and bug retained the blood meal and the microfilariae for at least 24 days. The blood and the microfilariae were digested at a slow rate but there was no development of the microfilariae noted. (5) The ceratopogonids, *Culicoides amamiensis, C. variipennis,* and to some extent *C. guttipennis,* ingested a blood meal con-

taining active, motile microfilariae. The blood was digested but the microfilariae made their way to the thoracic muscles to develop; 3d stage larvae were observed emerging from the proboscis of *Culicoides amamiensis* after 16 days.

Some caution is required in concluding that a particular experimental intermediate host is a vector of a filarioid parasite in the absence of definitive data on its feeding habits. For almost a year, *Culicoides* were not taken in great numbers from a monkey baited trap. Yet late in 1964, in the Hsiao-i area, considerable numbers were taken in just 1 hour.

Although the experimental and circumstantial evidence is strongly in favor of the hypothesis that the *Culicoides* is the arthropod involved in the natural transmission of *Macacanema formosana* from monkey to monkey, its acceptance leaves some questions with incomplete answers. However, because very large numbers of these insects congregate about and bite monkeys, there appears to be no incompatibility between the high infection rate in the monkeys of this area and the low infection rate in the observed *Culicoides*. It appears that although individual insects are inefficient, *Culicoides amamiensis*, taken as a population, is an efficient vector of *Macacanema formosana*.

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13. ABSTRACT

(1) Taiwan monkeys (Macaca cyclopis) were trapped and examined to determine the geographical distribution and foci of the filaria (Macacanema formosana). (2) The filaria-positive area was surveyed to determine the potential arthropod vectors. (3) Suspected intermediate hosts were experimentally fed on filaria-infected monkeys and the development of the filarial larvae has been recorded for the first time. (4) Studies were made to determine microfilariae density and periodicity. Ecologic factors are discussed. Some 22 species of arthropods experimentally exposed to infective blood meals from monkeys with infections of Macacanema formosana fall into five distinct groups based on the fate of the microfilariae from the peripheral blood; (1) The ceratopogonids, Culicoides anamiensis, C. variipennis, and to some extent, C. guttipennis, ingested a blood meal containing active, motile microfilariae. The blood meal was digested and the microfilariae made their way to the thoracic muscles to develop. The 3rd stage larvae were observed emerging from the proboscis of C. anamiensis after 16 days. The remaining groups were not as successful. (2) The argasid tick, Ornithodoros tartakovskyi, and the reduviid, Rhodnius prolixus, retained the blood meal for at least 24 days, but the microfilariae did not develop. (3) Stomoxys, Chrysops, and 5 genera (14 species) of culicids took a blood meal containing active, motile microfilariae that passed with the blood dejecta from the mosquito or fly in 48 to 120 hours with no development of the microfilariae. (4) Pedicinus eurygaster (lice) collected on filarial positive monkeys contained plasma or body fluids but no microfilariae were noted. (5) The one species of Simulium exposed would not take a blood meal. Culicoides is suggested as the natural vector of Macacanema formosana. A marked nocturnal microfilariae periodicity was shown.

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