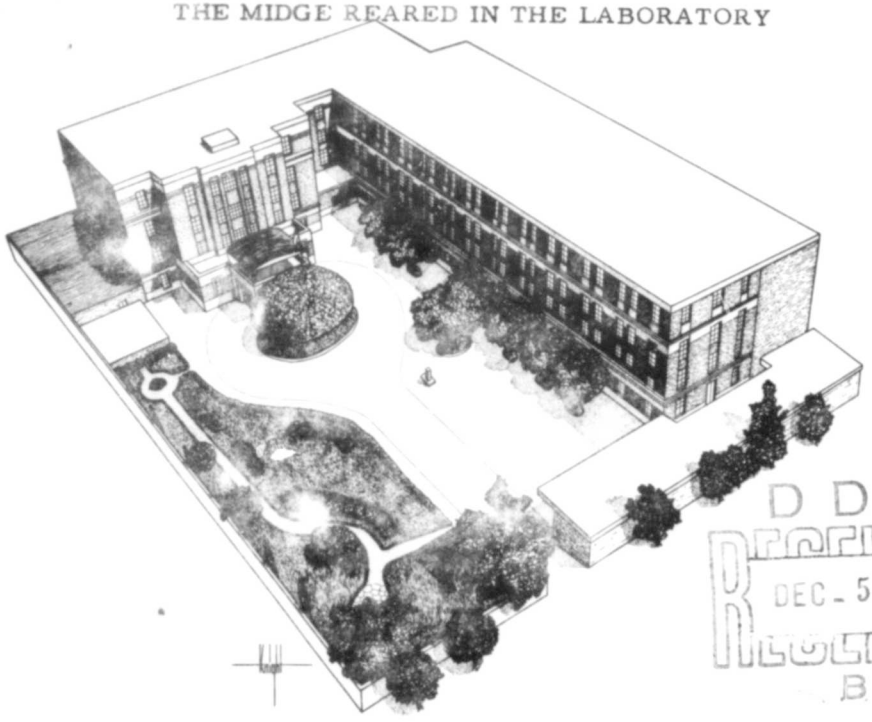
NAMRU-2

STUDY OF A BITING MIDGE, FORCIPOMYIA (LASIOHELEA) TAIWANA (SHIRAKI) (DIPTERA: CERATOPOGONIDAE) 1. DESCRIPTION OF THE COMPLETE LIFE CYCLE OF THE MIDGE REARED IN THE LABORATORY



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MEDICAL ECOLOGY DEPARTMENT
ENTOMOLOGY DIVISION
John H. Cross, Ph. D., Head

ADMINISTRATIVE INFORMATION

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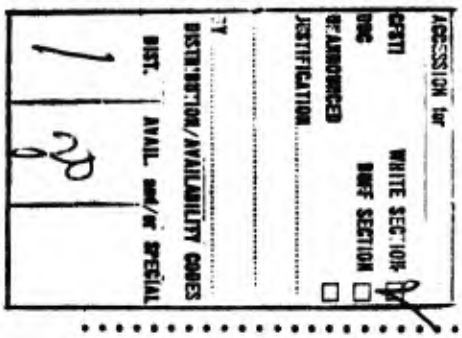

R. H. WATTEN, CAPT MC USN
Commanding Officer

STUDY OF A BITING MIDGE, FORCIPOMYIA (LASIOHELEA) TAIWANA (SHIRAKI) (DIPTERA: CERATOPOGONIDAE) I. DESCRIPTION OF THE COMPLETE LIFE CYCLE OF THE MIDGE REARED IN THE LABORATORY*#

by
Wesley K. C. Sun

Abstract: Forcipomyia (Lasiohelea) taiwana *females engorged with human blood were collected and placed in mist cages in the laboratory. Eggs and larvae were kept in a medium of laterite clay and yeast powder at a constant temperature of 28°C and relative humidity of about 80%. Eggs were deposited three or four days after feeding and hatching occurred within three days after oviposition. The larval stages required 16-18 days and the pupal stage 3-5 days for completion. The lif cycle from egg to adult ranged from 21 to 26 days. Descriptions of male and immature* F. (L.) taiwana *and larval dimorphism are presented for the first time.*

INTRODUCTION

The Chinese ideographs, "蟌蠓", for midges appeared in ancient Chinese literature about 2,500 years ago. The term is thought to have been used collectively for many swarming minute flies including biting midges of the Family Ceratopogonidae.[1] *Forcipomyia* (*Lasiohelea*) *taiwana*, "臺灣蟌蠓," was first described from Taiwan by Shiraki in 1913 based on females only.[2] Wu and Wu (1957) claimed to have isolated Japanese B encephalitis virus from *F.* (*L.*) *taiwana* in Fukien Province, China Mainland.[3] Liu *et al.* (1964) reported the breeding habits of *F.* (*L.*) *taiwana* in Fukien[4] and Lien and Lin (1966) observed the habits of *F.* (*L.*) *taiwana* in Hualien, Taiwan.[5] Sun (1961, 1966,1967) recorded the distribution of this species in Taiwan Proper and also on Quemoy Island and the Pescadores.[6,7,8]

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F. (*L.*) *taiwana* is widely distributed in the plains and foothills of Taiwan. Although it is commonly found below 100 meters elevation, its collection from Kappan Shan (460 M) suggests a higher vertical distribution.[9] The yearly appearance of *F.* (*L.*) *taiwana* is predomnaintly from April to October but occasional collections on clear cold days with air temperature around 10°C have also been made.[9]

F. (*L.*) *taiwana* bites during the day from 1000 to 1700 hours with a peak at 1400—1500. It invades dwellings through ordinary window screen and attacks man both indoors and outdoors mostly on the lower legs and occasionally on the arms. The bites inflicted by the midge are painful, usually producing intense irritation and itching. The reddish edematous spots on the skin remain for several days and may be the source of secondary infection through scratching. Nothing has been done about the possible role of this biting midge in disease transmission in Taiwan. Since Japanese B encephalitis is epidemic in Taiwan during the Summer season (Wang *et al.* 1962) [10] which coincides with the seasonal abundance of the biting midges, the physiology and pathophoricity of the midge should be investigated. After three years (1962—64) of field studies on *F.* (*L.*) *taiwana* in Taiwan and its adjacent islands, the author began to collect the living midges for study of the life cycle in the Entomology Laboratory at Tunghai University.

MATERIALS AND METHODS

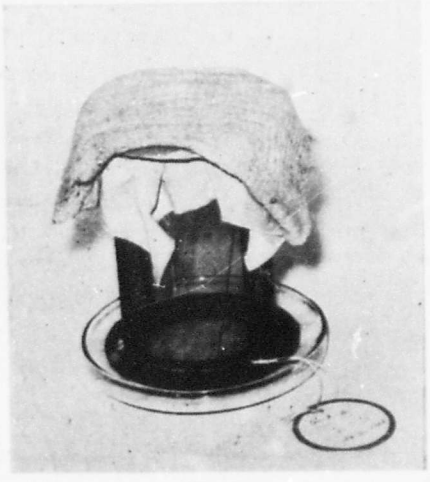


Fig. 1

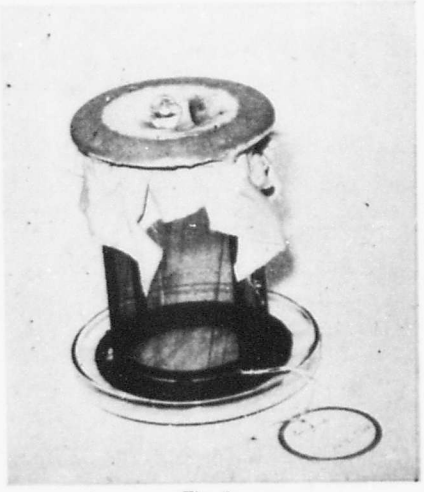


Fig. 2

Rearing cage for *F.* (*L.*) *taiwana* (Fig. 1). Wet cloth removed to show the sugar supply from a glass bulb inserted from the top (Fig. 2)

Adult female midges engorged with blood were collected by using human bait and brought back to the laboratory. They were kept in bronze or galvanized iron rearing cages, 11 X 15 cm, surrounded with nylon stocking material and covered by a moistened cloth to maintain a relative humidity of about 80% inside the cage (Fig .1.) The rearing cage was set in a flat culture dish with several layers of filter paper. As the gravid females will never oviposit on a dry substrate, the filter paper was kept constantly moistened. A glass bulb containing glucose solution (5%) was inserted in the top of the cage to supply sugar requirements for the midges. (Fig.2). Glucose was renewed three times per week. After oviposition, the eggs were collected and transferred to a petri dish with a larval culture mrdium of clay (laterite) and yeast powder, which was also kept constantly moistened. The petri dishes were put in a dark lowtemperatu-re-incubator with a temperature of 28°C and a relative humidity of about 80% for 16 to 18 days without disturbance. When the larvae became pupated, they were removed from the incubator and put in rearing cages for emergence.

RESULTS

Life Cycle—*F.* (*L.*) *taiwana* in the laboratory deposited eggs by the third or fourthday after feeding. Most eggs hatched within three days after oviposition. The larval stage required (16-18) days and the pupal stage (3-5) days. The duration of the life cycle of the midge, from egg to adult, was 21-26 days. Female midges usually die a few days after oviposition. The longest life span, however, of the laboratory reared females is 26 days.

Description of the gametes, of immature stages and of males of *F.* (*L.*) *taiwana.*

1. Egg and Spermato- an

Egg—banana-shaped, length about 0.5 mm and width at the widest part, about 0.13 mm; uniformly dark brown; usually 3—8 eggs forming an egg batch. First instars breaking out of egg in upper half and empty egg-shell resembling shoe. (Fig. 3).

Fig. 3. *F.* (*L.*) *taiwana*, Eggs (left) and egg-shells, enlarged. (right)

Spermatozoan—consists of a head about 8 X 3 microns and a long and vibratile tail about 130 microns (Fig. 4).

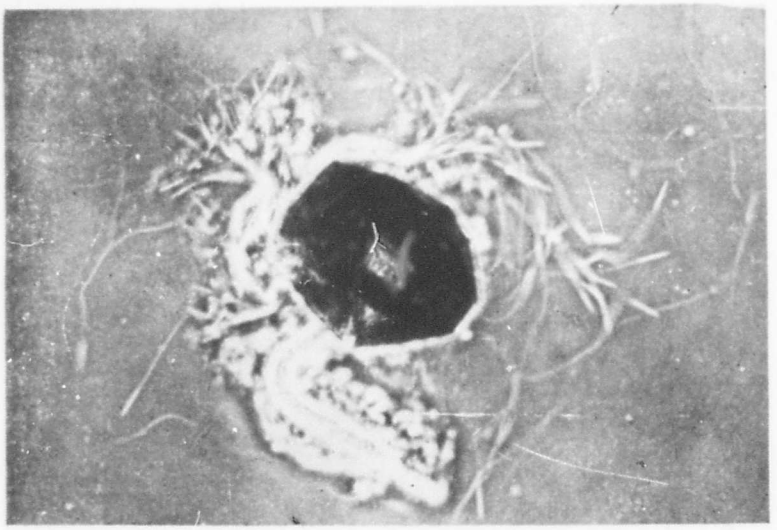


Fig. 4. *F.* (*L.*) *taiwana*, spermatheca and spermatozoa

2. First instar—body length, 0.55-0.63 mm, head length about 0.11 mm; colorless, transparent except yellowish-brown on head region and mouthparts, pseudopod hooklets dark. Body cylindrical, intersegmental constrictions smooth. Head, hypognathous, smoothly round at the profile end; antennae one pair, five pairs of hairs of various length and structure, the dominant and longest pair near eye base, 0.15 mm long (approximately 1/4 of whole body length) with spinulous sculpture. Antennae, rod-like arising from low tubercles. Eyespots round, prominent, bloody reddish in color immediately after emergence and turning gradually to dark brown as larva grows. Thorax-abdomen with minute dorsal simple hairs, three pairs of stronger lateral hairs on each segment except for last abdominal segment. Prothoracic pseudopod cylindrical, crowned with two rows of dark hooklets: 10 anterior bifid ones, and 10

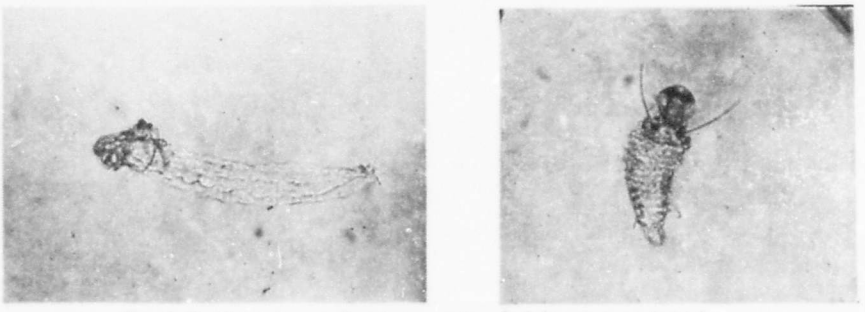


Fig. 5. *F.* (*L.*) *taiwana*, first instar: relaxed (left), contracted (right)

posterior sickle-like ones; ventral surface studded with minute spines in several transverse rows. Posterior pseudopod presenting, transverse ventral ridge with two rows of 18 black hooklets: 8 anterior sickle-shaped ones and 10 posterior bifid ones. (Fig. 5).

3. Full-grown instar—body length: 3.6-3.8 mm, head length 0.27 mm; color of head dark brown, mouthparts black, thorax and abdomen yellowish brown, hooklets of pseudopods dark. Intersegmental constrictions prominent. Head chaetotaxy: five hairs as follows, four simple pairs with two pairs in front of antennae, one pair behind and one pair beyond eyes, fifth pair situated beyond antennae, heavier and sculptured at end. Hairs on thorax—abdomen of first instar disappearing and replaced by large brownish club-like projections, four pairs per segment except for last abdominal segment; each projection arising from tubercle with a plumose ending, on last two abdominal segments each bearing a pair of median processes pointing backward. Prothoracic and posterior pseudopods with hooklets similar to those of first instar. (Fig. 6).

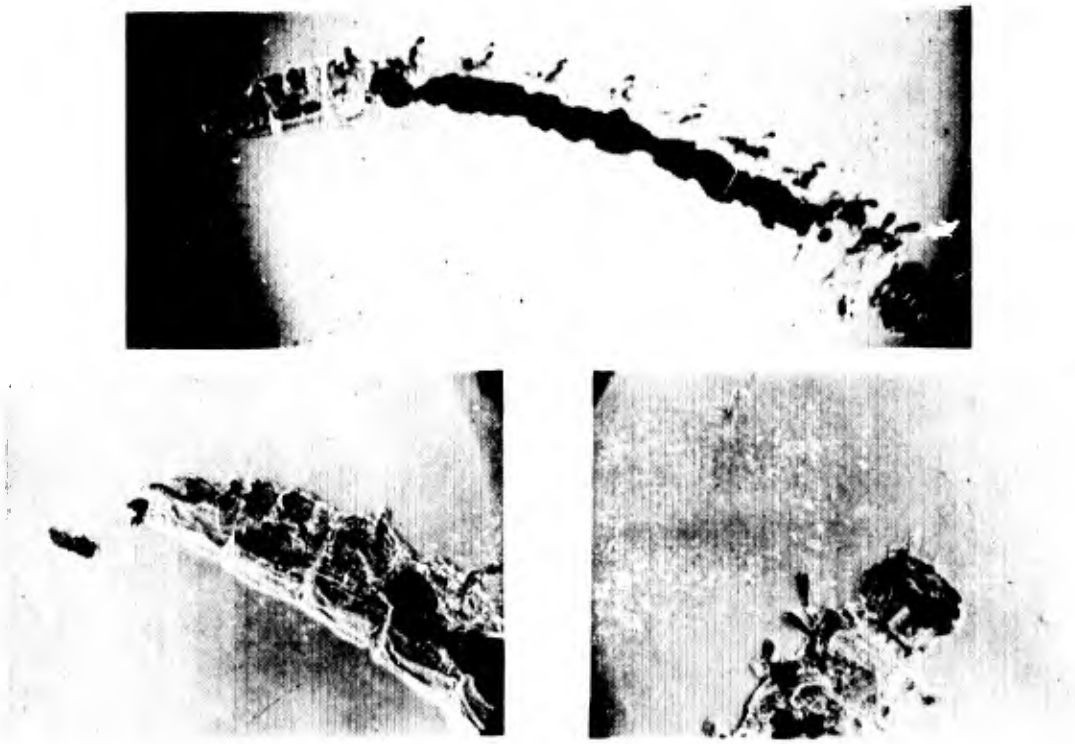


Fig. 6. *F.* (*L.*) *taiwana*, full-grown instar (phase contrast), upper: whole body; lower left: posterior region; lower right: head region

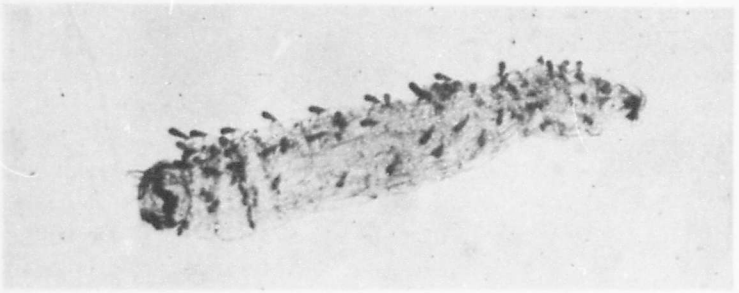
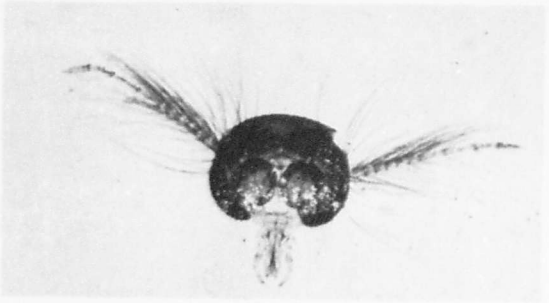


Fig. 6. *F.* (*L.*) *taiwana*, full-grown instar (continued), larval skin to show the segmental projections

4. Pupa—body length: 3.0-3.2 mm; color brownish, darker on thorax; retaining larval excuvia on posterior three segments; prothoracic horns rod-like with basal stems constricted at proximal ends, bearing 14 spiracular papillae around tip of each horn. Body gradually tapered, cuticular armature consisting of minute cones scattered along margin area of each segment. Hypopygial sheaths stout, triangular with pair of chitinized lateral lobes. (Fig. 8, D-E)



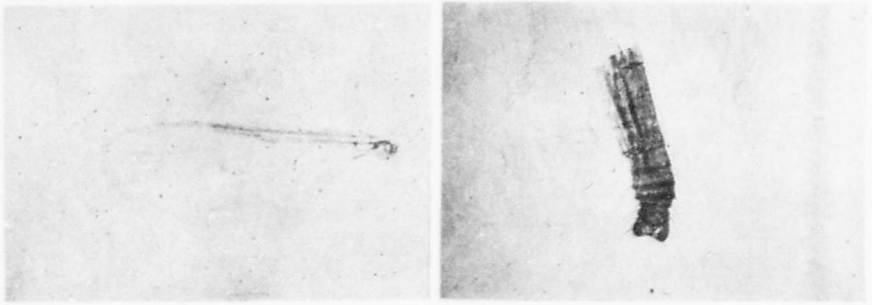
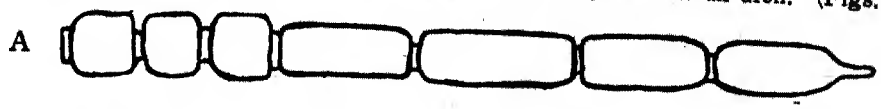


Fig. 7. *F.* (*L.*) *taiwana*, male, upper: head; lower left: wing; lower right: abdomen to show genitalia

5. Male—body about 1.4 mm long, wing about 0.84 mm by 0.27 mm. Head, dark brown; maxillary palpus with five segments in proportions of 7:14:14:8.5:15, segment 3 thickened at middle with many convergent spoon-like sensory projections in round pore. Antennae, plumose with last four segments long, proportion of distal seven segments, 12:12:12:25:30:24:27. Thorax, dark brown, legs yellowish brown; claws simple, empodium large, hind tibial comb with 12 or 13 basal and 8 apical bristles. Wing, pale yellowish, macrotrichia thick; costa ending slightly beyond middle of wing (33:59), first radical cell slit-like, second radical cell well formed, very narrow and longer than first one (6:10); fMCu beyond distal end of R_1. Halteres, pale yellow. Abdomen, brown. Genitalia, with 9th sternite square, basistyles cyclindrical, width about 2/3 of length; dististyles nearly straight, slightly curved preapically; aedeagus well chitinized, separated at middle, basal arms broad and triangular, apical parts divergent laterad with two pairs of horn-like processes; parameres smoothly joined in an arch. (Figs. 7; 8, A-C)



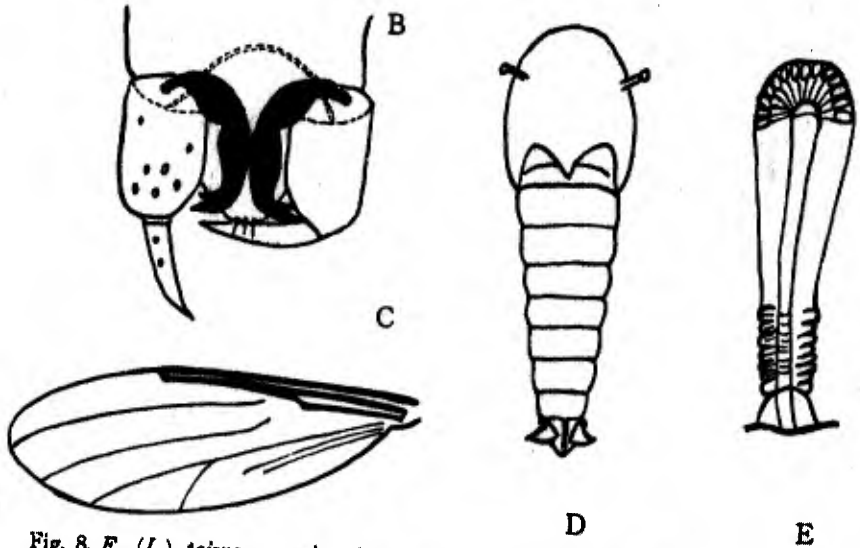


Fig. 8. *F.* (*L.*) *taiwana*, male: A. antenna, distal seven flagellar segments. B. genitalia. C. wing. pupa: D. whole body E. prothoracic horn

DISCUSSION

1. Dimorphism of instars—The first instars of *F.* (*L.*) *taiwana* are colorless with intersegmental constrictions inconspicuous, body setaceous and with one pair of hairs on the head being extraordinarily long. The grown instars are yellowish with intersegmental constrictions predominant, with only short hairs on head region, and hairless on body segments which bear four pairs of large brownish club-like projections on each segment. This dimorphic feature is similar in some respects to "hypermetamorphic" insects although in the latter two different larval forms, motile larva and sedentary larva, are always involved.

2. Genitalia—Genitalia of *F.* (*L.*) *taiwana* are allied somewhat to *F.* (*L.*) *ancoriformis* Tokunaga reported from Sabrosky, Caroline Islands, but are distinctly different from the allied species in the following points: the aedeagus provided with two pairs of horn ike apical processes and the paramere arch with median part round.[11]

3. Breeding habits—Liu *et al.* reported the breeding places of *F.* (*L.*) *taiwana* from Fukien and described them as sandy soil with some humus, damp and partially or entirely shaded. Wang *et al.* (1965)[12] collected *F.* (*L.*) *taiwana* larvae from soil at Ta-Tu-Shan, Taichung by means of the floating technique of Liu but using saturated $MgSO_4$ solution as suggested by Ladell (1936)[13] and Kettle and Lawson. (1952)[14] instead of saturated salt solution. Lien and Lin discovered the breeding places of *F.* (*L.*) *taiwana* at Hsio-Lin-Hsiang, Hualien Hsien by using funnel-traps set up in damp places of the infested area and many emerging adults were collected; however, soil investigations for immature stages were not successful.

The laboratory-rearing study reveals that *F.* (*L.*) *taiwana* breeds in damp soil; most larvae live within the top soil and full-grown larvae move up to the surface for pupation. Wet soil, and/or soil enriched with organic matter is not suitable for rearing. All the immature stages are negative to light and, on the other hand, the adults are positively phototropic.

4. Vector of Japanese B encephalitis?—Wu and Wu reported that they had isolated Japanese B encephalitis virus from *F.* (*L.*) *taiwana* at Fukien.[3] Since their midges were collected from human bait, there remains the question as to whether or not the virus isolated was from carrier's blood. S ince *F.* (*L.*) *taiwana* is very abundant and widely distributed in Taiwan, a further study of the relationships of the virus, midge, and man is needed.

SUMMARY

Forcipomyia (*Lasiohelea*) *taiwana* is widely distributed in Taiwan. It is noxious and has been reported from Fukien as being the vector of Japanese B encephalitis virus. This midge was first described by Shiraki in 1913 based on females only. Males as well as immature stages were not known to him, nor have they been reported by others since. Female midges engorged with blood were collected by the author and reared in the Entomology Laboratory at Tunghai University, Taichung, Taiwan. They deposited eggs by the 3rd or 4th day after feeding. Most eggs hatched within three days after oviposition. The larval stage lasted 16—18 days and pupal stage 3—5 days. The duration of the life cycle of the midge, from egg to adult, was 21—26 days. Female midges usually die a few days after oviposition. The longest life span of the laboratory reared females, thowever, is 26 days. The structure of the immature and male *F.* (*L.*) *taiwana* as well as the dimorphism of the larvae are described.

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臺灣蠕蠓之研究

一、實驗室培育與生活史之完成

孫克勤

臺灣蠕蠓在本省分布甚廣，北起宜蘭，南迄屏東，均有其存在。東部之花蓮，中部之南投以及東海所在之大度山，尤爲著名之孳生區。此蟲形體微小全長不過一毫米，每年五至十月間常白晝成群飛出，襲擊人體，刺吮血液，咬螫之處，發刺痛奇痒，且致皮膚炎腫，歷久不消。在我國福建省更有傳遞日本腦炎之報導，危害人類健康非淺。

一九一三年，日人素木得一於「臺灣總督府農事試驗場特別報告」第八卷首次記載臺灣蠕蠓，唯僅限於雌體之敍述，至於本種之生活史包括雄體，幼蟲之構造與習性，尙缺完整之報告。

作者以身爲餌，將吸血之蠕蠓採集而飼養於實驗室中，觀察其卵巢發育、產卵、孵化、生長與變化。發現其完全之生活史，約歷廿一至廿六日。計：卵--幼蟲二至三日，幼蟲--蛹十六至十八日，蛹--成體三至五日。有關臺灣蠕蠓之雄體、雌雄配子、幼蟲、蛹之構造與習性以及幼蟲發育過程之同種二形現象，本文曾有論述。

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13. ABSTRACT

Forcipomyia (Lasiohelea) taiwana females engorged with human blood were collected and placed in mist cages in the laboratory. Eggs and larvae were kept in a medium of laterite clay and yeast powder at a constant temperature of 28°C and relative humidity of about 80%. Eggs were deposited three or four days after feeding and hatching occurred within three days after oviposition. The larval stages required 16 - 18 days and the pupal stage 3 - 5 days for completion. The life cycle from egg to adult ranged from 21 to 26 days. Descriptions of male and immature F. (L.) taiwana and larval dimorphism are presented for the first time.

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