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TWO NEW MOSQUITO SPECIES FROM A PITCHER PLANT OF MT. KINABALU, SABAH, MALAYSIA: *CULEX RAJAH* AND *TOXORHYNCHITES RAJAH* (DIPTERA: CULICIDAE)

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Abstract: *Culex* (*Culiciomyia*) *rajah* n. sp. and *Toxorhynchites* (*Toxorhynchites*) *rajah* n. sp. were collected from the pitcher plant *Nepenthes rajah* at high elevations of Mt. Kinabalu, northern Borneo. *Cx. rajah* n. sp. is characterized by: adults with a dark stripe on upper parts of the pleura and a distinct pale basal band on terga II—VII; pupa with single 4-VIII setae and broad paddles; and larvae with short setae, 7-C and 8-P, a slender siphon with usually 5 pairs of 4—6 branched 1-S tufts, and long gills. *Tx. rajah* n. sp. is characterized by: adults with a combination of long rm crossvein of wings, absence of brown fusiform scales on mesokatepisternum of thorax; and absence of well-developed caudal tufts on terga VI—VIII; pupae with setae 11,12-CT single, 1-II single, 6-I—VII long and single, and broad shape of paddles; and larvae with 2-branched setae 1-III,IV, single 4-VI, single 6-P and 7-M, single 11-V, and a long siphon with 2-branched 1-S.

INTRODUCTION

During mosquito surveys in Malaysia in 1986, mosquitoe larvae were collected from water in pitchers of *Nepenthes rajah* Hooker f. on Mt. Kinabalu, Sabah, Malaysia. According to Kurata (1976), this species of pitcher plant is endemic to this mountain and it occurs at high elevations (1,650—2,650 m). Together with several known mosquito species, larvae of two unknown species belonging to subgenus *Culiciomyia* of the genus *Culex* and to the genus *Toxorhynchites,* respectively, were also collected.

The former was initially thought to be *Cx. shebbearei* because of a previous record by Edwards (1931) and a citation by Barraud (1934), Knight and Stone (1977) and Beaver (1983). According to the redescription of *Cx. shebbearei* by Sirivanakarn (1977), the larval characters illustrated for this species are notably different in morphology from the newly collected *Culiciomyia* larvae from Mt. Kinabalu.

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Another species of *Culiciomyia* recorded from the same mountain is *Cx. javanensis,* and larvae of these recorded species are similar to each other. However, adults of *Cx. javanensis* are distinctly different from those of *Cx. shebbearei* and from the present new materials, and there is no fear of confusion in identification, as will be discussed later. Results of further taxonomic examinations on the new materials led to the conclusion that this must be a new species, although it has been misidentified as *Cx. shebbearei* for many years.

From the morphology of adults, pupae and larvae of *Toxorhynchites,* Steffan and Evenhuis (1985) classified 36 known Asian species into 7 species-groups. The newly available specimens of *Toxorhynchites* reared from the collected larvae, however, does not coincide with any of the known species, indicating a possibility of another new species.

Materials and Methods

Mosquito larvae were collected by the author together with Dr. Motoyoshi Mogi on 24 September 1986 on Mt. Kinabalu at elevations of about 1,600 m to 1,800 m. Larval collection was carried out carefully in order not to destroy any pitcher plants, with the permission of and regulated by the Department of Saba National Park, Kota Kinabalu, Saba, Malaysia, some of whose staff members accompanied us.

Some of the larvae collected were used to study biochemical systematics by means of electrophoresis (Tsukamoto *et al.,* 1989), but to obtain pupae and adults for further identification, some larvae were reared under laboratory conditions in the University of Malaya, Kuala Lumpur, Peninsular Malaysia. Probably due to sudden changes of environmental conditions during the transportation, such as shaking, temperature and/or air pressure, some adults of *Toxorhynchites* failed to emerge from pupae. Both larval and pupal skins of the holotype of *Cx. rajah* n. sp. were lost in an accident. Therefore, description of pupa and larva was based on whole body paratypes preserved in a 70% ethanol solution.

The descriptions of the new species are based mainly on the format used by Bram (1967) and by Sirivanakarn (1977) for *Culex,* and by Steffan and Evenhuis (1985) for *Toxorhynchites.* With slight modifications, tables for pupal and larval chaetotaxy are also based on the form used by Evenhuis and Steffan (1986) and Tanaka *et al.* (1979).

Abbreviation of generic and subgeneric names of mosquitoes followed the proposal by Reinert (1985).

Descriptions

Culex (Culiciomyia) rajah, n. sp.

(Figures 1a, 2; Table 1)

The 4th instar larvae are characterized by a combination of 1) a short 7-C hair, 2) a very short 8-P hair with 2—4 branches, 3) a slender siphon with usually 5 pairs of 4—6 branched hair tufts, and 4) long gills. In the adult, thorax shows an indistinct dark stripe at upper pleura and a dark spot in middle of sternopleuron. Abdominal terga II—VII have broad pale basal bands.

MALE. *Wing*: 3.6—3.8 mm. *Head*: Proboscis entirely dark with scanty median ventral tuft; palpus dark, about 1.2 times length of proboscis, segment III at the apical half with a distinct ventral row of about 5 long translucent scales. *Thorax*: Integument of pleuron tan

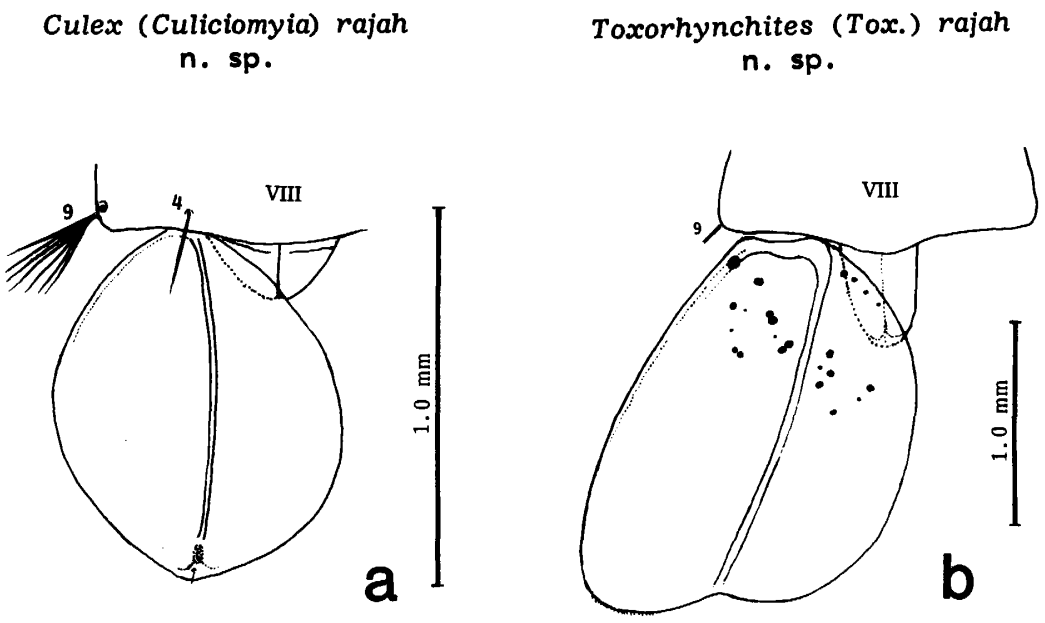

Figure 1 Paddles of pupae of two new species.
a: *Culex rajah*, b: *Toxorhynchites rajah*.

with a typical dark upper stripe, and another isolated dark spot on middle of sternopleuron. *Wing* with cell R2 (forked cell) 1.7—1.9 times length of vein r2+3 (stem). *Abdomen*: Terga II—VII dark with distinct broad basal pale band covering an area about equal to dark portion of each tergum II—V or more than half of each tergum VI—VII. *Male genitalia*: Not described.

FEMALE. *Wing*: 4.1—4.3 mm. Generally similar to male. *Head*: Segment IV of palpus longer than (or about) twice length of segment III. *Thorax*: Wing with cell R2 2.35—2.5 times length of vein r2+3. *Abdomen*: Terga II—VII with pale basal bands covering less than half length of each tergum.

PUPA. Seta 4-VIII single, 9-VIII 3—8 branched. Paddle, 0.9 mm length, broad and deformed oval, index (length/width) about 1.2, apex slightly projected (Figure 1a).

LARVA (Figure 2, Table 1). *Head*: Width about 1.3 mm. Antennal tuft 1-A 13—15 branched, attached at 2/5 length of the shaft from base; seta 1-C fine and simple; 4-C single; 5-C 3,4 branched; 6-C 3 branched; 7-C short (about 1/2 length of 5,6-C), 4 branched; 8,10-C single; 9-C short, 2—3 branched. *Thorax*: Seta 0-P minute, about 9 branched; 1-P double; 2,3-P single; 4-P double; 5,6-P single; 7-P 3—4 branched; 8-P very short (1/6 to 1/4 length of 7-P) forked with 2—4 branches, scarsely single; 14-P single. Seta 1-M shorter than 3,4-M. *Abdomen*: Setae 6-I,II long, 3—4 branched, 6-III,IV,V,VI long, 2—5 branched; 7-I long, single or 2 branched; 7-II,III,IV,V very short, 2—6 branched; 1-I,II,III,VI minute or short, 1-IV,V long, single or 2 branched, 1-VII single, about the same as length of segment VII, 1-VIII 3,4 branched; 2,4-VIII single; 3-VIII 6—8 branched; 5-VIII double; comb consisting of 45—60 narrow fringed scales. *Saddle* shorter; caudal margin with many strong spicules; 1-X single; 2,3-X single slightly less than length of siphon or gills; 4-X 4 pairs, weak and short, especially a precratal pair shortest and usually single, other 3 pairs 2—4 forked. *Siphon* slender, 1.7—1.8 mm length (n=17), not inflated in middle but gradually tapering to apical; siphon index

Table 1 Chaetotaxy of the 4th instar larvae of *Culex* (*Culiciomyia*) *rajah* n. sp.

Seta No.	Head	Thorax			Abdomen							
	C	P	M	T	I	II	III	IV	V	VI	VII	VIII
0	—	9m	—	—	—	—	—	—	—	—	1m	—
1	1 s	2 L	1m	2m	3m	2-3m	2 s	1-2 L	1-2 L	1-2 s	1	3-4
2	—	1 L	2m	3 s	1m	1m	1m	1-2 s	1-2 s	1m	1m	1
3	1m	1	1-2 s	1-2m	2-3 s	2-3 s	2m	2-3 s	2-3m	1 s	2-3 s	6
4	1m	2	1m	3-4	5 s	1-2 s	1-3	1-2 s	2-3 s	2-3 s	1 s	1
5	3-4 L	1 L	1	3-4m	2m	2m	1-2m	1-2m	2m	2m	1-2m	1
6	3 L	1 L	1 L	1 s	3 L	3-4 L	2-3	3 L	2-3 L	2-3 L	5 s	2
7	3-4 s	3-4	2 L	6-8 L	2 L	2-4	5 s	5-6 s	2-5 s	1 s	1-2m	
8	1 s	2-4 s	5 L	6-8m	—	1 s	1 s	1 s	1 s	1 s	5m	1-A 13-15
9	2-3 s	1 s	6	5	1-5m	1 s	5 s	1 s	1 s	1 s	1-2m	1-S 4-6 (5 pairs)
10	1 s	1 s	1 L	1 L	1	1	1 s	1 s	1 s	1 s	1m	1-X 1
11	3-4 s	2-3m	2m	1m	1m	2m	1-2 s	2m	2m	2m	1 s	2-X 1L
12	2-3m	1 L	1 L	1 s	1	12m	1m	1m	1m	1 s	1 s	3-X 1L
13	2-3 s	6-8m	6-8m	3m	1 s	9m	3 s	2-4 s	3 s	10-12m	2-4m	4-X 1-4 (4 pairs)
14	1m	1 s	3-5m	—	—	—	—	—	—	—	—	1m
15	3-4m	—	—	—	—	—	—	—	—	—	—	—

L: long, s: small, m: minute, —: absent or not detected.

Culex (Culiciomyia) rajah n. sp.

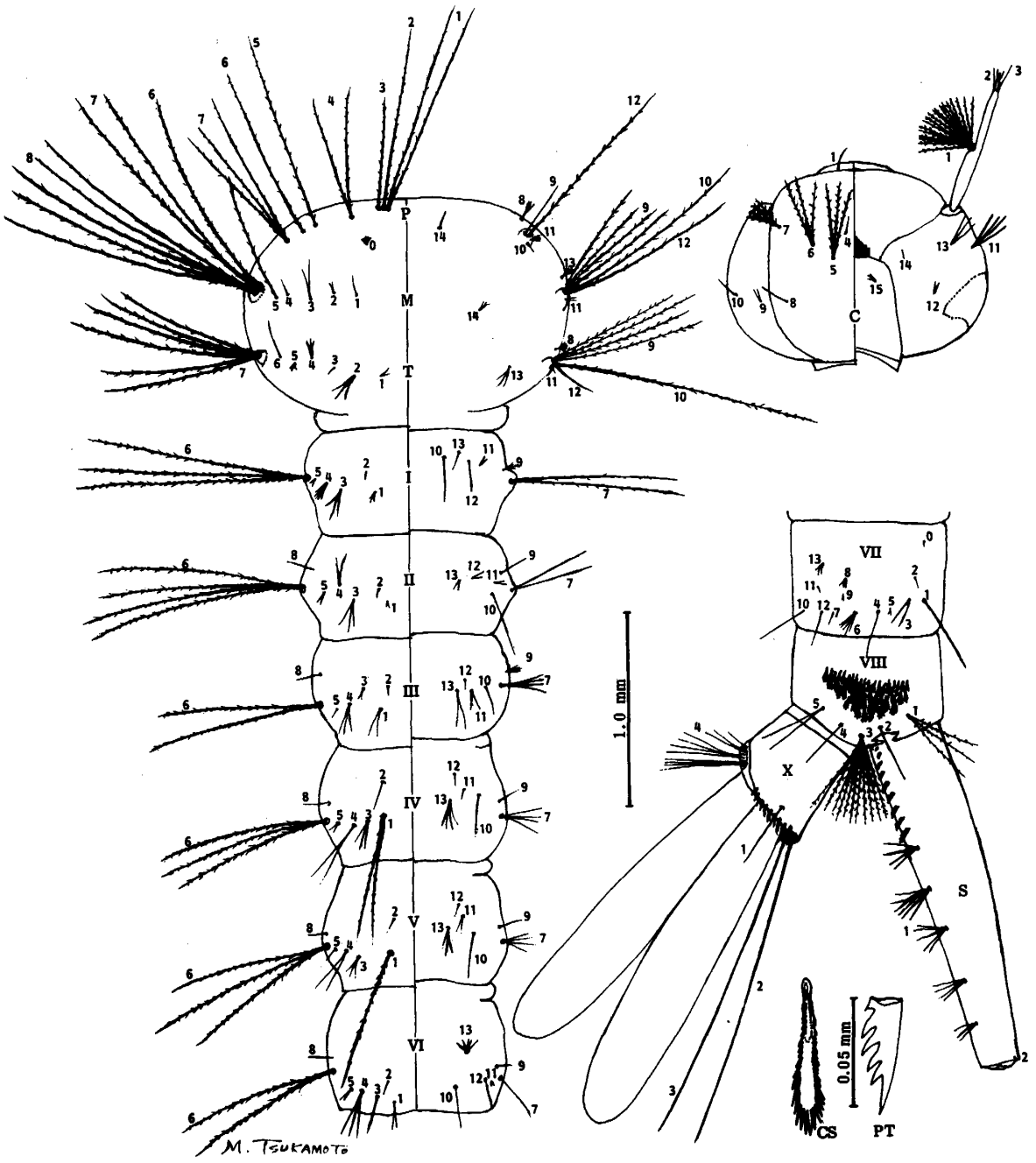

Figure 2 Larva of *Culex rajah*.
A: antenna, C: head, P: prothorax, M: mesothorax,
T: metathorax, S: siphon, CS: comb scale, PT: pecten.

4.0—5.5, 1-S mostly 5 pairs (occationally 4 or 4.5 pairs), individual tuft hair 4—6 branched and as short as 0.4—0.5 of the width of siphon at the point of attachment; pecten teeth 10—12, each tooth short and wide with a strong distal spine and 4—5 strong lateral denticles. Upper anal gill about the same as length of siphon, lower gill slightly shorter than upper gill. Larval chaetotaxy is given in more detail in Table 1.

TYPE DATA. Holotype male reared from a 4th instar larva collected on 24 September 1986 by M. Tsukamoto, from a pitcher of *Nepenthes rajah* in Mt. Kinabalu, Sabah, Malaysia. Paratypes: 5 males, 6 females reared from larvae, and 15 larvae collected by M. Tsukamoto; 10 pupae (4 females, 5 males and 1 skin with a half-emerged male from it) and 30 larvae collected by Dr. M. Mogi on the same date at the same place. Holotype and some of paratypes will be deposited in the National Museum, Tokyo, Japan.

DISTRIBUTION. Known only from the type locality, Mt. Kinabalu, Sabah, Malaysia.

BIONOMICS. Larvae of *Cx. rajah* n. sp. occur in pitchers of *Nepenthes rajah* at high elevations in association with larvae of *Culex* (*Lophoceraomyia*) *jenseni* (De Meijere), *Tripteroides* (*Rachionotomyia*) sp. No. 2 of Mattingly (1981), *Uranotaenia* (*Pseudoficalbia*) *moultoni* Edwards, and *Toxorhynchites* (*Toxorhynchites*) *rajah* n. sp. Body surface of most larvae are covered by *Vorticella*-like protozoa. Nothing is known of their adult biology, habitat, or medical importance as a vector of diseases.

TAXONOMIC DISCUSSION. About 30 species of mosquitoes belonging to subgenus *Culiciomyia* of the genus *Culex* are known in Asian countries (King, 1946; Sirivanakarn, 1973, 1977; Sirivanakarn and Kurihara, 1973; Knight and Stone, 1977; Toma *et al.*, 1984; Apiwathnasorn, 1987; Harrison, 1987). From North Borneo 6 species are so far known of this subgenus: *Cx. fragilis* Ludlow, *Cx. nigropunctatus* Edwards, *Cx. shebbearei* Barraud, *Cx. spathifurca* (Edwards), *Cx. papuensis* (Taylor), and *Cx. javanensis* Bonne-Wepster.

The original record of *Cx. shebbearei* by Edwards (1931) is based on the collection by Mr. H. M. Pendlebury in 1929 from a giant pitcher plant, *Nepenthes rajah,* on Mt. Kinabalu as follows: "The adults reared from these larvae were *Culex shebbearei,* Barraud, a species which had been found on one previous occasion only, when Barraud obtained larvae in the water in a hollow tree in the eastern Himalayas". At that time neither taxonomic discussion nor basis for this identification was given by him. However, this record was cited by Barraud (1934), Knight and Stone (1977), Beaver (1983) and Apiwathnasorn (1986) without any further evidence, although Sirivanakarn (1977) mentioned that the record of *Cx. shebbearei* from Borneo was doubtful.

According to the redescription based on the type specimens and illustration of *Cx. shebbearei* by Sirivanakarn (1977), larvae of this species have 4 pairs of siphonal hair tufts (1-S), "first proximal pair double; second proximal pair triple; 2 distal pairs double, 1.5—2.0 times as long as siphonal width at point of attachment". However, larvae of *Cx. rajah* n. sp. possess 5 pairs (occasionally 4 pairs in either one or both sides) of the siphonal hair tufts, and each hair is short (about 1/2 width of siphon at point of attachment) and has a different number of branches (4—6). Relatively long anal gills and broad shape of pecten teeth are also different from those illustrated by Sirivanakarn (1977) for a larva of *Cx. shebbearei.*

From the morphology of larvae described above and from these situations about the present species, therefore, it seems more likely to conclude that the species is a new species which has been misidentified as *Cx. shebbearei* for a long time, rather than to think that both *Cx. shebbearei* and *Cx. rajah* n. sp. are living in pitchers of *Nepenthes rajah* on Mt. Kinabalu.

Culex javanensis was also recorded from Mt. Kinabalu by Sirivanakarn (1977), but this species can be readily distinguished from the new species in having fewer number (4 pairs) and fewer branches (2—3) of siphonal tufts in larvae, and by absence of basal pale bands on terga in adults.

Some important larval characters among known east and southeast Asian species of *Culiciomyia* are compared in Table 2, where *Cx. azurini* (with a single short seta 1-A and an incomplete saddle) and *Cx. termi* (with an unusually long siphon) are not included because they are easily distinguishable from other members by their peculiar morphology (Toma *et al.*, 1984; Thurman, 1955). *Cx.* (*Thaiomyia*) *dispectus* Bram and *Cx.* (*Thaiomyia*) *hainanensis* Chen are also excluded, although Harrison (1987) proposed to include the subgenus *Thaiomyia* into the subgenus *Culiciomyia*. *Culex harrison* Sirivanakarn, *Cx. ceramensis* Sirivanakarn and Kurihara, and sometimes *Cx. nailoni* King and Hoogstraal also have 5 pairs of the siphonal tufts, but they differ from *Cx. rajah* n. sp. in having double branched 2,3-P, 3,4-branched 4-P and 5-VIII, respectively. In addition, larvae of both *Cx. ceramensis* and *Cx. nailoni* have double 5,6-C hairs, and the shape of the siphon in the former species is not slender but more or less elliptical. *Culex harrisoni* resembles the present new species in having a minute hair 8-P, long gills, branch numbers of several setae and tufts, but distinguishable by length of anntenna (longer in *Cx. rajah* n. sp.), branches of 7-C (fewer in *Cx. rajah*), 2,3-P and 5-VIII hairs.

Regardless of subgenera, only limited members of the genus *Culex* have minute or short hair 8-P: for example, *Cx.* (*Cux.*) *alis, Cx.* (*Eum.*) *tenuipalpis, Cx.* (*Lop.*) *curtipalpis, Cx.* (*Lop.*) *pholeter, Cx.* (*Lop.*) *uniformis,* etc. Especially in the subgenus *Culiciomyia,* only *harrisoni, rajah* n. sp., *dispectus* and *hainanensis* fall into this category in Asia. Therefore, the length of the hair 8-P must be a good feature quite helpful in confirming a particular species.

In adults, *Cx. rajah* n. sp., *Cx. harrisoni, Cx. shebbearei, Cx. viridiventer,* and *Cx. bailyi* are rather similar, all having dark marks or a distinct stripe on upper pleura of the thorax and distinct pale basal bands on terga II—VII of the abdomen. However, these basal bands are narrow in *Cx. shebbearei* but broad in *Cx. rajah* n. sp. Table 3 compares taxonomically important characters in adults of several known species in Asia.

Toxorhynchites (Toxorhynchites) rajah, n. sp.

(Figures 1b and 3; Tables 4,5)

Adults are characterized by a combination of 1) absence of well-developed lateral tufts on terga VI—VIII, 2) absence of brown fusiform scales on mesokatepisternum, 3) absence of pale greenish lateral scale portion of scutum, and 4) long rm crossvein of wings. Larva is characterized by 1) a single and long 6-P hair, 2) bristles 7-P, 13-M and 7-T 2 branched and 3) long siphon and saddle.

MALE. *Wing*: 5.5—6.5 mm. *Head*: Proboscis dark without pale or lighter marking, maxillary palpus-I dark purple, with a small spot of silvery-white scales at the apex of segment I, segments II—V without dorsal lighter marking. *Thorax*: Scutum with metallic greenish blue scales, without pale green lateral stripe zones, anterolateral portion of mesokatepisternum bare. *Wings* with long rm crossveins. *Legs* practically without white scales on all tibiae and tarsi except faint lighter scales on baso-ventral portions of midtarsi I—II. *Abdomen*: Terga bluish purple, with yellowish lateral markings on I, and small baso-lateral light marks on terga III—VII. Terga VI—VIII with only poor black lateral setae not forming

Table 2 Comparison of taxonomically important larval characters of (*Culiciomyia*) spp. in Asia

Mosquito species	Siphon index	1-S pair	Branch number of seta										Remark
			1-S	5-C	6-C	1,	2,	3-P	4-P	7-P	5-VIII	1-X	
spiculothorax	3.5-4	4	3-5	3	3	2	1	2	2	2	1	1	thorax spiculated
harrisoni	4	5(4)	3-5	3-5	3-4	2	2	2	2	3-5	1	1	8-P minute
rajah n. sp.	4-5.5	5(4)	4-6	3-4	3	2	1	1	2	3-4	2	1	8-P very short
lampangensis	4-4.5	4	4-5	2	3-4	3	1	2	—	3	1	1	siphon swollen; 1-C dark
papuensis	4-5.5	4-5	4-10	3	3-4	3	1	3	2	3	2	2-3	siphon swollen; 1-C stout
sasai	4.6-5.8	4(5)	2-6	2-4	2-4	2	1	2	2	2-3	1-2	1	saddle emarginate around 4-X
ryukensis	4.7-5.9	3	2	3-4	3-4	2	1	1-2	2	2	2	1	long gills
pallidothorax	4-6	4	2-6	2-3	2-4	2	1	2-3	2	2-3	2	1	siphon swollen
ceramensis	5	5	4-6	2	2	1-2	1	2-3	3-4	2-3	2	3	siphon swollen
nailoni	5	4-5	3-4	2	2	—	—	—	—	—	3-4	1	1-S tufts long
shebbearei	5.5	4	2-3	4	3	—	—	—	—	2-3	1	1	1-S tufts long
viridiventer	5.5	4	3-5	2-3	2-3	2-3	1	2	2	3	2	2	1-M, 1-III minute or short
barrinus	5-6	3-4	3-4	2	2	2	1	3	2	2	3	3	siphon swollen, with dark band
fragilis	5-6	3	2-3	5-8	5-8	1	1	1	2	2-3	2	1	1-III—VII short and branched
spathifurca	5-6	3	1	3	3	1	1	1	2	2	3	1	1-IV,V,VII long and single; gills shorter
kyotoensis	6-7	4	1-4	2-5	2-5	2	1	2-3	2	2	1-3	1	1-M,1-III as long as 3-M,3-III
bailyi	6.5-7.5	3	1-2	3-4	3-4	2	1	2	2	2	2	1	8-P, 1-M long
javanensis	7	4(3)	2-3	3	3	2	1	1	2	2	2	1	1-S tufts long
thurmanorum	7-8.5	3	1	5-6	4-5	2	1	1	2-3	2	4	1	setae stellate
ramalingami	9-10	4	4-6	5-6	5-6	1	1	3-5	5	3	3	3	1-S tufts very long; 1-M,T strong, 4 branched
scanloni	9.5-10.5	3	2	3	2	1	1	1	2	2	2	1	pecten tooth with stout, rounded basal barb
nigropunctatus	9-11	3	1-3	3-5	3-4	1	1	1	1-2	2-3	1-2	1	siphon with false joint; 1-I,II distinct
pullus	10-14	3	2	3	3	1	1	1	2	2	2	1	siphon with false joint; 1-I,II minute

—: Information not available from published description or illustration.

Larva unknown: *Cx. bahri, Cx. fuscicinctus, Cx. delfinadoae* and *Cx. tricuspis.*

Table 3 Grouping of adult mosquitoes of subgenus (*Culiciomyia*) spp. in Asia

Abdomen Terga II—VII	Thorax (Upper pleura)	
	Without dark mark	With dark marks or a stripe
Without pale band	*bahri, javanensis, fragilis, nailoni, scanloni*	*ceramensis, papuensis, spathifurca*
With pale spot mark	———	*dispectus, fuscicinctus*
With pale lateral mark	*ramalingami*	———
With pale basal band	*lampangensis*	*ryukensis, kyotoensis, shebbearei, bailyi, viridiventer, harrisoni, rajah* n. sp., *barrinus, pullus, pallidothorax, thurmanorum*
With pale joint band	———	*nigropunctatus*
With pale apical band	*azurini, tricuspis, delfinadoae*	———
With dark basal band	*termi*	———

Adult unknown: *spiculothorax,* ——— : No case in point.

either well-defined lateral or caudal tufts. *Male genitalia*: Not described.

FEMALE. *Head*: Apex of maxillary pulpus silvery-white scaled. *Thorax*: Generally similar to males. Forelegs without distinguishable marks. Midlegs with light scaled marks on basal 1/2 to 2/3 of T-I, almost all T-II yellowish white except dark scales at dorso-apical portion, T-III, IV, and V entirely dark scaled. Hind T-II with light scaled marking at baso-ventral portion.

PUPA (Figure 1b, Table 4). Setae 10-CT 3 forked, 11,12-CT single; 1-I multibranched (about 10), 2,4,5,9-I single, short, 3,6-I single, long; 1,3,5-II single (length of 5-II about 1.6 times length of 1-II), 2-II single, minute, 4-II 3 branched, short; 1,3,5-III single, long, 4-III 3 single short; 1,5-IV single, long, 2,3-IV single, short, 4-IV 2 branched short; 1,5-V single, long, 2,3-V short, single; 4-V 3 branched short; 1,2,4-VI single, short, 3-VI 2 branched short, 5-VI single, long; 1,2,3,4-VII single, short, 5-VII single, long; 4,9-VIII single, short. *Paddle* (Figure 1b) broad and subrhomboid-ovate, length 2.4 mm, width 1.4 mm; with scattered dark spots or subbasal mottling line; slightly emarginated at apex of midlib; with minute marginal spicules near apex of lobe.

LARVA (Figure 3, Table 5). *Head*: Integument brown without special dark dorsal mark; setae 9,10-C single. *Thorax*: Satae 1,2,3-P single, 1-P longer than 2-P on the same tubercle, 3-P isolated, very short, 4-P isolated, forked with 7 branches, 5-P stout, single, 6-P long, single, 7-P strong, 2 branched, 10-P long, single; 1,2-M isolated and very short, 3—7-M single on the same sclerotized plate, 6-M stout, 7-M minute, 10-M single, long, 13-M stout 2 branched; 1-T long, single, 2-T short, single, 3,4-T small, separated, 6-T stout, single, 7-T stout, 2 branched, 8-T minute, 4 branched, 9,13-T stout, single, 10-T slender, single. *Abdomen*: Setae 1-I,II,V,VI,VII single, 1-III,IV 2 branched, 3,4-I strong, 2 branched; 2-I minute, single and isolated; 2-II—VI short, isolated; 4-I,II 2 branched; 4-III—VII single; 10-I short, single; 10-II—V, 2 branched; 10-VI small, single; 10-VII short; single; 11-I—IV 2 branched; 11-V—VII single; 12-I minute, branched; 12-II—VII minute or short, single; 13-I-VI long, single; 1-VIII small, single and

Table 4 Chaetotaxy of the pupae of *Toxorhynchites* (*Toxorhynchites*) *rajah* n. sp.

Seta No.	Cephalo-thorax	Abdomen							
		I	II	III	IV	V	VI	VII	VIII
0	—	—	—	—	—	—	—	1m	1m
1	1L	10	1	1L	1L	?	1m	1	—
2	1m	1m	1m	1m	1m	1m	1m	1m	—
3	1m	1L	1L	1	1	1m	2m	1	—
4	1m	1m	3-4	1	2	2-3	1	1	1
5	1m	1m	1L	1L	1L	1L	1L	1L	—
6	1	1L	1L	1L	1L	1L	1L	1L	—
7	1L	1	1	1	1m	1m	1m	1m	—
8	1L	—	1m	1m	1m	1m	1m	1m	—
9	1	1	1m	1m	1m	1m	1m	1-2m	1
10	3	—	1	1	1	1m	1m	1m	—
11	1	—	1m	1m	1m	1m	1m	1m	—
12	1	—	—	—	—	—	—	—	—

L: long, m: minute, — : absent, ? : lacked.
Specimen examined: a single pupal skin associated with a larval skin.

isolated; 2,3-VIII small, single; 4,5-VIII stout, single. *Siphon* long, 1.3 mm, siphon index about 2.5; 1-S stout and 2 branched. *Saddle* narrow, about same as length of siphon, 2 times length of basal width; 1-X single, stout; 2-X 6 branched, one of them longer than others (about 2 times length of the shortest); 3-X long (about 2.8 times length of siphon), 3 branched; 4-X 6 pairs on grid, their length ranging from 1 to 1.4 length of saddle. Gills very short.

TYPE DATA. Holotype male reared from a larva collected in Mt. Kinabalu, Sabah, Malaysia, by M. Tsukamoto on 24 September, 1986. Paratype 1 male, 2 females, 2 pupal skins, and 1 larval skin, collected by M. Tsukamoto and M. Mogi on the same date.

DISTRIBUTION. Known only from the type locality, Mt. Kinabalu, Sabah, Malaysia.

BIONOMICS. Larvae of this species were collected in water of *Nepenthes rajah,* in association with larvae of *Cx.* (*Cui.*) *rajah* n. sp., *Cx.* (*Lop.*) *jenseni,* and *Ur.* (*Pfc.*) *moultoni.* Adult do not suck blood but other biology is unknown.

TAXONOMIC DISCUSSION. In Asia at least 40 spp. of mosquitoes belonging to the genus *Toxorhynchites* are counted. Steffan and Evenhuis (1985) assigned 36 known species (at that time) into 7 species-groups by the combination of various morphological characters. Based on one female from Mt. Kinabalu, at about 1,300 m elevation, *Tx. pendleburyi* was described by Edwards (1930). This species possesses well-developed caudal tufts on terga VI, VII and VIII, belonging to the *splendens* species-group of Steffan and Evenhuis (1985). Since the present new species has poor caudal tufts, this cannot be *Tx. pendleburyi.* The *acaudatus* group is characterized by 1) absence of well-developed caudal tufts, 2) long rm crossvein of wings, 3) presence (in some species) of brown fusiform scales on anterolateral region of mesokatepisternum in adults, 4) presence (in some species) of subbasal mottling on paddles of pupae, and 5) pitcher plant dwelling immatures. The present new species, *Tx. rajah,* have long rm crossvein of wings, without distinct lateral tuft on terga VI—VIII; this tends toward *acaudatus* group, but the new species does not have brown fusiform scales in anterolateral

Toxorhynchites (*Tox.*) *rajah* n. sp.

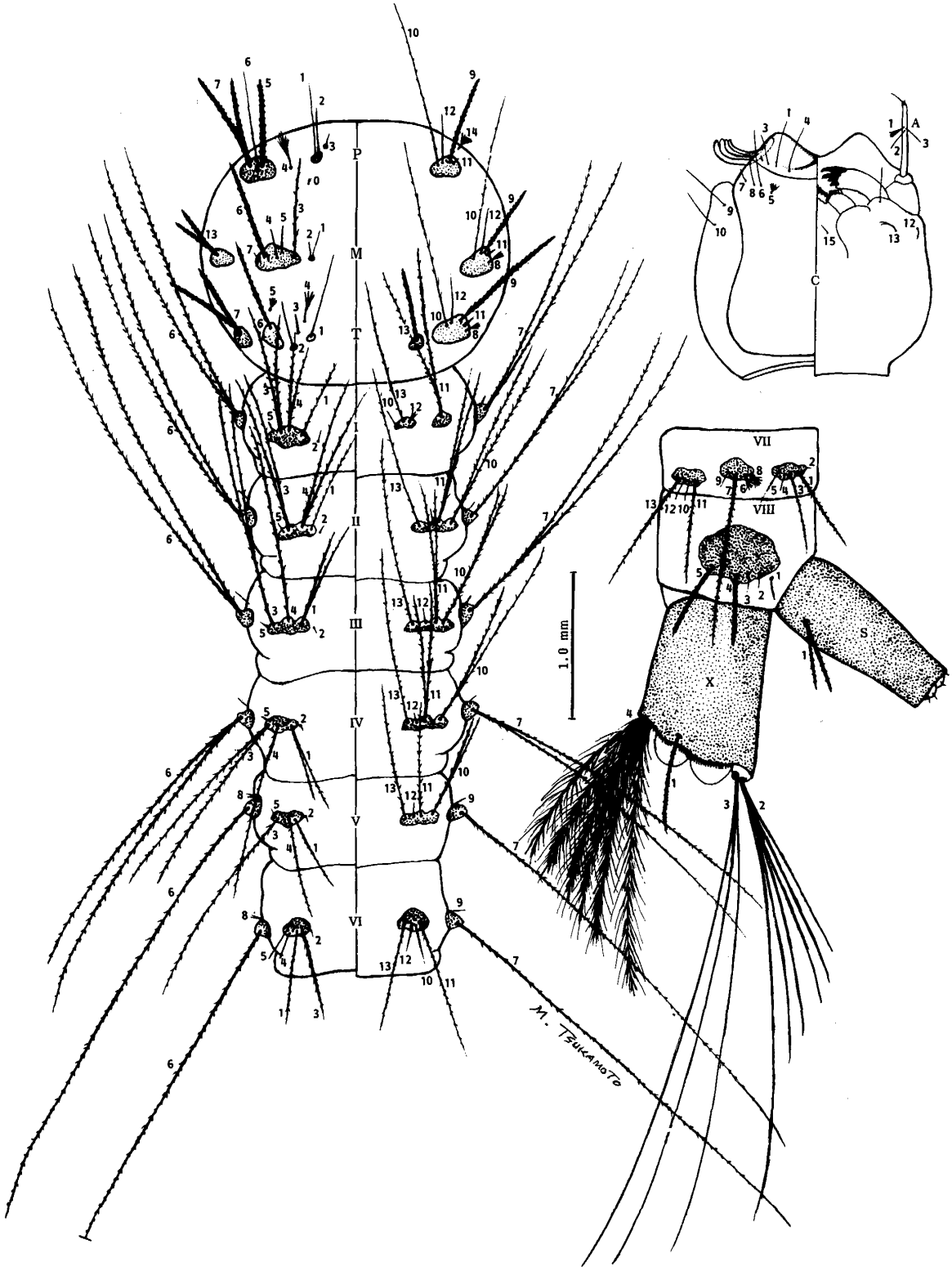

Figure 3 Larva of *Toxorhynchites rajah*.
A: antenna, C: head, P: prothorax, M: mesothorax,
T: metathorax, S: siphon.

Table 5 Chaetotaxy of the 4th instar larva of *Toxorhynchites* (*Toxorhynchites*) *rajah* n. sp.

Seta No.	Head	Thorax			Abdomen							
	C	P	M	T	I	II	III	IV	V	VI	VII	VIII
0	1m	2	—	—	—	?	?	?	?	?	?	?
1	1	1	1	1	1	1	2	2	1	1	1	1
2	—	1	1m	1	1m	1m	1m	1m	1m	1m	1m	1m
3	1	1m	1	1m	2L	2L	2L	2L	1-2L	1	1	1m
4	1	7	1	4	2	2L	1L	1L	1	1	1m	1S
5	5m	1S	1	4m	1m	1m	1m	1m	1m	1	1	1S
6	1	1	1S	1S	2L	2L	2L	2L	1L	1L	5m	
7	1m	2S	1m	2S	2L	2L	2L	2L	1L	1L	1L	1-A 4
8	1	?	5m	4m	—	1m	1m	1m	2m	2m	7m	1-S 2S
9	1	1S	1S	1S	1m	1m	1m	1m	1m	1m	1m	1-X 1S
10	1	1L	1L	1L	1m	2L	2L	2L	2	1	1	2-X 6L
11	—	1m	1m	1m	2L	2L	2L	2L	1L	1L	1L	3-X 3L
12	1	1	1	1	3m	1m	1m	1m	1m	1	1	4-X 1L
13	2	—	2S	1S	1L	1L	1L	1L	1L	1	1L	(6 pairs)
14	—	6m	—	—	—	—	—	—	—	—	?	—
15	1m	—	—	—	—	—	—	—	—	—	—	—

L: large, S: stout, m: minute, —: absent, ?: not detected.
Specimen examined: a single larval skin associated with the pupal skin.

region of thorax. More recently Evenhuis and Steffan (1986) have described *Tx. angustiplatus* from the Malay Peninsula as a new species which also does not have the brown fusiform scales. Pupae of the latter species, however, can be easily distinguished from those of the present new species because *Tx. rajah* has paddles with subrhomboid-ovate shape and single setae 11,12-CT and 1-II (like in *Tx. nepenthis*) instead of elongate paddles of *Tx. angustiplatus* pupae and the branched setae in question. Pupa of *Tx. rajah* is also distinguished from that of *Tx. nepenthis* by a branched seta 10-CT and a single long seta 6-VII. In addition, larvae of the present new species are unique by showing elongate siphon and saddle, and by chaetotaxy completely different from that of *Tx. nepenthis* by long and stout setae 5,7,9-P, 6,9,13-M, 6,7,9,13-T, 4,5-VIII and 1-S. Number in branches of these setae also different. Larval chaetotaxy of *Tx. rajah* is shown in Table 5 in more detail.

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マレーシア，キナバル山のウツボカズラより採集されたクシヒゲカとオオカの2新種

塚本　増久

1986年度文部省科学研究費による海外調査でマレーシアの蚊相を調べた際，ボルネオ島キナバル山の高所に自生する，食虫植物ウツボカズラの壺から数種の蚊幼虫を採集する機会があったので，その系統分類学的研究を行った。それらのうちのクシヒゲカの1種は1929年に採集され，*Culex shebbearei* として記録されていたものであったが，これを精査したところ東ヒマラヤ原産のこの学名の種とは全く異なり，新種であることが判明したので，*Culex* (*Culiciomyia*) *rajah* オウサマクシヒゲカ（新称）と命名して詳しい記載を与えた。また，同種のウツボカズラから発生するオオカも未記載の新種であることが確認されたので，これも *Toxorhynchites* (*Toxorhynchites*) *rajah* オウサマオオカ（新称）と命名し，成虫，蛹，幼虫などの形態について記載を行った。学名および和名は，これらの蚊が採集された巨大なウツボカズラ *Nepenthes rajah* の種小名（王様の意）に基づくものである。なお，同じ水域には *Culex* (*Lophoceraomyia*) *jenseni, Uranotaenia* (*Pseudoficalbia*) *moultoni, Tripteroides* (*Rachionotomyia*) sp. No. 2 などの蚊幼虫も発生していた。

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MANAGEMENT AND UTILIZATION OF ORPHAN DRUGS AGAINST PARASITIC INFECTIONS IN RECENT 7 YEARS IN JAPAN

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Abstract: Since many important antiparasitic drugs are not registered in Japan, a research group, for the past 7 years, has managed the use of non-registered orphan drugs. Initially there were 16 orphan drugs under control of the group, these were as follows; chloroquine, Fansidar, Fansimef, quinine (iv), primaquine, mebendazole, praziquantel, thiabendazole, quinacrine, dehydroemetine (iv), pentamidine, Pentostam, suramin, stibophen, nifurtimox and pyrimethamine. Of these drugs, Fansidar, thiabendazole, mebendazole and praziquantel were recently registered. The number of cases treated with these drugs was nearly one thousand (920). The number of malaria cases treated in this group was 201, corresponding to 22% of the total cases treated with orphan drugs. Mebendazole was used in 210 cases, praziquantel in 163, thiabendazole in 120, quinacrine in 125, pentamidine in 67, dehydroemetin in 28, Pentostam in 5 and suramin in 1. Most drugs were evaluated as effective in their antiparasitic actions but severe adverse reactions occurred at relatively high incidences.

INTRODUCTION

Tropical diseases are not endemic in Japan because of its geographical location in the temperate zone. In addition, helminthic diseases, which prevailed in Japan for 2 decades after World War II, have reduced incidence due to a change from use of night soil to chemical fertilizer in agriculture, and due to improvements of public health and environmental hygiene. Recent economical growth in Japan has created an urbanized life style for most people. On the other hand, an increase of international relations has caused an importation of parasitic diseases. In connection with the latter problem, effective antiparasitic drugs are necessary.

At the start of the present study, only a limited number of drugs effective against parasitic infections were available on the market. Those were pyrantel pamoate, pyrvinium

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pamoate, metronidazole, tinidazole, diethylcarbamazine, bithionol and quinine chloride (po, powder), paromomycin and so on. Other drugs were not registered in Pharmacopoeia Japonica, so-called "orphan drugs". Although many antiparasitic drugs have been developed in recent years and used widely in the world, pharmaceutical companies were not willing to register the drugs in Japan, on account of the cost in application for approval, limited business benefits and the occurrence of adverse reactions to these drugs. In order to collect clinical data on the orphan drugs, the Ministry of Health and Welfare in Japan (MOHW) set up a research group for orphan drugs in 1980 and the members stored, managed and studied 16 different drugs. The clinical trials began in 1981 and the orphan drugs have been supplied for use for 7 years. The present study deals with the results of the clinical trials and the present management of orphan drugs against parasitic diseases in Japan.

METHODS

Our research group managed non-registered drugs and advertised the drug list. Each member of the group stored a set of drugs for distribution in accordance with the legal responsibility of the member. An individual physician who wishes to use a particular drug, contacts any member of the research group by telephone. The member sends out the requested drug along with prepared forms and guidelines for use of the drug. The doctor sends back a form of official request and an informed consent signed by the patient. The doctor is obligated to make a report of the results of the trial. All drugs used in this study were purchased from pharmaceutical companies in foreign countries.

The following drugs have been managed in stock: antimalarial drugs; (1) chloroquine phosphate (Aralen, Resochin), (2) sulfadoxine-pyrimethamine (Fansidar), (3) mefloquine hydrochloride in Fansidar (Fansimef), (4) quinine dihydrochloride (parenteral, Quinimax), (5) primaquine phosphate (Primaquine); and other antiparasitic drugs, (6) mebendazole (Vermox), (7) praziquantel (Biltricide), (8) thiabendazole (Mintezol), (9) quinacrine hydrochloride (Atabrine), (10) dehydroemetine dihydrochloride (Dehydroemetine), (11) pentamidine isothionate, pentamidine methanesulfonate (Lomidine), (12) sodium stibogluconate (Pentostam), (13) suramin (Germanin), (14) stibophen (Fouadin or Neo-Antimosan), (15) nifurtimox (Lampit) and (16) pyrimethamine (Daraprim).

RESULTS

Clinical data on the use of orphan drugs have been collected for 7 years from April, 1981 to March, 1988, and are shown in Table 1 for malaria cases, in Table 2 for cases treated with mebendazole, praziquantel and thiabendazole and in Table 3 for parasitic infections treated with other anthelmintics.

Antimalarial drugs

Clinical trials of antimalarial drugs were carried out in 189 cases, out of which 158 cases were reported to this group as shown in Table 1. 106/158 (67%) cases were *Plasmodium vivax* infections, 50 (32%) were *P. falciparum,* 2 (1%) were *P. malariae,* and in the other 26 cases malaria species were not identified. The relationships of the species and antimalarial drugs are shown in Table 1.

Table 1 The number of clinical trials in malaria cases by use of chloroquine, Fansidar and quinine in 7 years

Species of plasmodium	Antimalarial drugs				
	Ch[a]	Fa[b]	Ch & Fa	Qu[c] & others[d]	Total
Plasmodium falciparum	16	7	9	18	50
Plasmodium vivax	61	36	9		106
	(53)[e]	(32)	(7)		(92)
Plasmodium malariae	1	1			2
		(1)			(1)
unable to identify	31	11	1		43
	(12)	(4)	(1)		(17)
Total	109	55	19	18	201
	(65)	(37)	(8)		(110)

a : chloroquine phosphate.
b : Fansidar (pyrimethamine-sulfadoxine).
c : quinine (parenteral, quinine dihydrochloride).
d : other antimalarial drugs including chloroquine (6 cases), Fansidar (6 cases) and both drugs (2 cases).
e : ( ) indicates the number of cases in which primaquine was used.

Primaquine was used in 92 cases (90%) of vivax malaria to prevent recurrence, and as a result the recurrence occurred in 3 cases originating from Southeast Asia (Thailand). Relapse was also seen in 2 patients with falciparum malaria. They had been treated with chloroquine or/and Fansidar, then quinine was given orally based on clinical evidence of drug-resistant malaria. One fatal case of falciparum malaria occurred due to delayed initiation of malaria therapy.

Adverse reactions were suspected in 3/42 cases (7%) treated with chloroquine (skin eruption, photophobia and liver injury) but no adverse reactions were reported with Fansidar.

Mebendazole

Mebendazole was administered in 210 cases in which 126 (60%) were *Trichuris trichiura* (whipworm) infection (Table 2). In most patients with trichuriasis, counts of eggs per gram (EPG) were at low levels except for one individual with a high EPG over 5,000. In 53/55 (96%) cases, EPG decreased after treatment. Adverse reactions were suspected in 5/75 (7%) cases. Abdominal pain and skin eruption were the reported reactions.

This drug was also used for treatment of trichinosis during an epidemic in 1981 in which about 60 persons were suspected of infecting the disease. The outbreak occurred in patrons of a restaurant in Yokkaichi in Mie Prefecture, where raw bear meat, possibly imported, was served to about 400 persons (Yamaguchi, 1983). There were two other epidemics in Japan; 15 out of 20 hunters in Aomori Prefecture in 1974 (Yamaguchi *et al.*, 1975) and 12 out of 94 persons who were served raw bear meat of local origin in Sapporo, Hokkaido in 1979 (Ozawa *et al.*, 1981; Tebayashi *et al.*, 1981).

Gnathostomiasis caused by *Gnathostoma spinigerum* and *G. hispidum* from raw fish sometimes occurs in Japan. The source of a recent infection with *G. hispidum* was imported

Table 2 Parasitic diseases and effects of mebendazole, praziquantel and thiabendazole

Drugs and illness	No. of cases	Collected cards	Effect (%)	Prognosis* C	R	D	U	Side effect Yes	No
Mebendazole									
trichuriasis	126	96	85 (89%)	73	1		22	5	70
trichinellasis	55	4	2 (50%)	3			1		4
gnathostomiasis	13	4	3 (75%)	3			1	1	3
multi. echinococcus[a]	11	2	0 (0%)	1			1		2
others[b]	5	2	2 (100%)		1		1		2
Total	210	108 (51%)	92 (85%)	80	2	0	26	6 (7%)	81
Praziquantel									
clonorchiasis	87	40	36 (90%)	38	2			11	25
paragonimiasis									
westermani	17	11	8 (73%)	7	2		2	2	6
miyazakii	4	1	1 (100%)				1		1
fasciolasis	6	1	1 (100%)	1					1
schistosomiasis									
japonica	29	9	3 (33%)	4		2	3	2	1
haematobia	4	1	0 (0%)				1		
tape worm[c]	8	7	6 (86%)	6			1		6
others[d]	8								
Total	163	70 (45%)	55 (79%)	56	4	2	8	15 (27%)	40
Thiabendazole									
strongyloidiasis	106	75	65 (78%)	53	5	1	16	20	55
Toxocara canis	7	2	2 (100%)	1			1	2	
gnathostomiasis	5	3	1 (33%)	2			1	2	1
others[e]	2	1	1 (100%)	1				1	
Total	120	81 (68%)	69 (85%)	57	5	1	18	25 (31%)	56

a : *Echinococcus mutilocularis*
b : others include *Angiostrongylus* (2 cases), *Toxocara canis* (1 case), larval migrans (1 case) and *Capillaria philippinensis* (1 case).
c : *Diphyllobothrium latum* (fish tapeworm, 7 cases), *Taenia solum* (pork tapeworm, 2 cases) and *Taenia saginata* (beef tapeworm, 1 case).
d : others include *Metagonium yokokawai* (5 cases) and *Sparganum mansoni* (3 cases).
e : others include *Capillaria philippi.* (1 case) and *Hymenolepis nana* (1 case).
* : C; cured; R; recurrent, D; died, U; unknown.

loaches from a neighboring Asian country (Ishii, 1983). Mebendazole was effective in reducing complaints due to subcutaneous or visceral larval migration.

Mebendazole was examined for treatment of *Echinococcus mutilocularis* infection, alveolar hydatid disease (AHD). Clinical cases were reported from endemic regions in the Japanese island of Hokkaido.

Praziquantel

Praziquantel was used in 163 cases, of which clonorchiasis (liver fluke) made up 87

Table 3 Parasitic diseases and drug effects (continued)

Drugs and illness	No. of cases	Collected cards	Effect (%)	Prognosis* C	R	D	U	Side effect Yes	No
Quinacrine									
giardiasis	42	26	25 (96%)	17			8	3	16
tapeworm									
fish[a]	34	30	21 (70%)	11			10	17	12
beef[b]	9	7	6 (86%)	5			2	4	2
type unknown	40	18	4 (22%)	4			16	4	9
Total	125	81 (65%)	56 (69%)	37			36	28 (42%)	39
Pentamidine									
P. carinii[c] pneumonia 67	67	41 (61%)	20 (49%)	5		29	7	12 (38%)	20
Dehydroemetine									
hepatic amebiasis	28	14 (50%)	8 (57%)	9		1	4	3 (25%)	9
Pentostam									
Kala-Azar	3	2	2 (100%)	1		1		1 (50%)	1
leishimaniasis	2	0							
Suramin									
trypanosomiasis	1	1	1 (100%)	1					

a : *Diphyllobothrium latum.*
b : *Taenia saginata.*
c : *Pneumocystis carinii.*
* : C; cured, R; recurrent, D; died, U; unknown.

(53%) (Table 2). In clonorchiasis, the cure rate was 90% at a dosage of 25 mg/kg, given three times a day for 2 days.

In paragonimiasis (lung fluke) caused by *Paragonium westermani* and *P. miyazakii* (rare), praziquantel was shown to be effective with about a 70% cure rate.

The effect of praziquantel on *Schistosoma japonicum* infections could not be evaluated properly because new infections have ceased to occur in Japan since 1976 and all cases in this study were at chronic stages. Four cases of *S. haematobium* (Japan Overseas Cooperation Volunteers) originated from Egypt.

Adverse reactions to praziquantel were suspected in 15/54 cases (27%); including eruption (5 cases), drowsiness (4), abdominal pain (4) and diarrhea (2).

Thiabendazole

Thiabendazole was used in 120 cases, mainly for strongyloidiasis (Table 2). Infections with *S. stercolaris* occur regionally in the Southwest Islands including Okinawa Island. The cure rate was 87% but recurrence of the illness was often observed. Adverse reactions reported with thiabendazole were abdominal discomfort (13 cases), drowsiness (5) and eruption (2).

Other drugs

Quinacrine was used for the treatment of giardiasis (42 cases) and it provided a high rate

of cure. The drug was effective in recurrent cases after treatment with metronidazole. In one case of giardiasis, toxic reactions including liver damage and eruption occurred after treatment with quinacrine. Quinacrine was also used as an anthelminthic drug in 83 cases of tape worm infection.

Cases of hepatic amebiasis and *Pneumocystis carinii* pneumonia are increasing in number in Japan, resulting in increased uses of dehydroemetine and pentamidine, respectively.

A Japanese woman was infected with African trypanosoma, *Trypanosoma rhodesiense* and fortunately cured by administration of suramin (Negishi *et al.*, 1984).

Newly registered drugs

The present study describes the success of this first step toward effective administration of orphan drugs in this country. Among 16 orphan drugs listed previously, 4 drugs, thiabendazole, Fansidar, mebendazole and praziquantel were subsequently registered and are now available on the market. Two other drugs, chloroquine and pentamidine, are under consideration by the Central Pharmaceutical Affairs Council (CPAC) in MOHW. The data collected by this research group were widely utilized during evaluation for registration of these drugs.

Discussion

Adverse reactions of the drugs studied by our group occurred often in this country, partly on account of metabolic differences of Asian races including Japanese from other races. In other instances, intoxication with chloroquine, chinoform and salidomide occurred by the result of prolonged treatment or large dosages. The pharmaceutical bureau in MOHW and pharmaceutical companies were legally accused of fostering intoxication with these drugs. The court trials concerning chinoform and salidomide were resolved by a compromise between plaintiffs and the supplying companies, however, court trials regarding chloroquine are still pending.

The reason why MOHW is also in litigation together with the supplying companies is because in Japan the approving organizations or review committees are charged with the same responsibilities as the accused when untoward effects result from the approved products. Because of the above legal liabilities and on-going court trials, CPAC recently has been careful in reviewing and approving new drugs. The same issues exist for decisions by ethical review committees in Japan for new medical trials on human subjects.

Due to careful attention to discussion and approval of drugs by CPAC, only data from preclinical studies strictly following the standardized methods defined by CPAC and carried out in Japan were the subject of consideration in the past. Nevertheless, data from preclinical studies conducted in foreign countries by methods, essentially equal to the Japanese standards, were recently considered valid by the Japanese CPAC.

Phase 1 and 2 clinical trials were conducted in normal and infected Japanese, respectively, by our research group. With the possibility of using preclinical studies from foreign countries and the data from clinical trials conducted by our group, pharmaceutical companies were encouraged and decided to apply for registration of a few orphan drugs and to offer these drugs for commercial sale as an action of social contribution, even though commercial merits would not be expected. Because of the above stated difficulties, 12 drugs still remain as orphan drugs in Japan.

In the USA, the Centers for Disease Control (CDC) Drug Service presently manages 9 drugs: diloxanide furoate (Furamide), dehydroemetine, nifurtimox (Lampit), sodium stibogluconate (Pentostam), melarsoprol (Mel B), suramin, bithionol, ansamycin, and quinine dihydrochloride (parenteral) as orphan drugs. Out of these 9 drugs, 5 are common to our orphan drugs. Our supply system for these drugs is incidentally the same as that in the USA, although our organization is composed of researchers without assigned staffs.

There are few reports dealing with clinical trials using newly developed antiparasitic drugs in Japan (Tanabe and Tanaka, 1986; Tanabe *et al.*, 1987). Data in the present study may reflect a new trend in parasitic infections in Japan because it is difficult for individual physicians to obtain antiparasitic orphan drugs from any other source by themselves.

In the present study, the total number of cases is not as large as that seen in tropical counties. However, in accordance with the increase in international travelers, parasitic diseases have become an important problem even in developed countries. From this point of view, the present study demonstrated an effective way for our country to provide orphan drugs needed to treat parasitic diseases.

Many problems have remained unsolved regarding parasitic diseases in our country. *Echinococcus multilocularis* infection occurs locally in Hokkaido and about 260 cases have been detected by clinical and pathological observations. According to recent reports based on serological surveys, echinococcus infections have been increasing in number (Takahashi, 1986). Amebiasis, giardiasis, strongyloidiasis and *Pneumocystis carinii* pneumonia are also increasing in number in association with STD or as opportunistic infections. Treatment of these diseases remains a problem and should be solved by developing new types of drug.

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寄生虫病薬の7年間の治験成績の検討
—稀用薬の入手緩和を求めて—

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昭和55（1980）年に発足した厚生省「輸入熱帯病の治療薬に関する研究」班では，寄生虫病の治療に稀用される薬剤の入手緩和を計ることを検討してきた。わが国の薬事で承認されていない寄生虫薬で，海外においては一部で既に使用されている16種の薬剤，すなわちファンシダール，クロロキン，プリマキン，キニーネ(注射液)，ファンシメフの抗マラリア薬とそれ以外のミンテゾール，メベンダゾール，プラジカンテル，ペンダミジン(注射液)，アテブリン，デヒドロエメチン（注射液)，ペントスタム（注射液)，スラミン，スチィボフェン，ランピット，ピリメタミンを選定して輸入し，治験用に供給して臨床試験を実施してきた。昭和56（1981）から62（1987）年までの7年間に，920症例(マラリア患者では2種以上の治験薬が投与されても1症例として集計した）に薬剤を配布してきた。症例の内訳は201例（22%）がマラリア患者で，マラリア以外の投薬症例数はメベンダゾールが210例，プラジカンテル163例，アテブリン125例，ミンテゾール120例，ペンタミジン67例，デヒドロエメチン28例，ペントスタム5例，スラミン1例である。このうち治療カードの回収できた症例について，有効性と副作用を検討して概略をまとめた。なお，今日ではファンシダール，ミンテゾール，メベンダゾール，プラジカンテル，ペンタニジンの承認が済んでいる。

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STUDIES ON EFFECTS OF PAROMOMYCIN SULPHATE, BITHIONOL, MEBENDAZOLE AND FLUBENDAZOLE IN THE TREATMENT OF MICE INFECTED WITH PLEROCERCOIDS OF *DIPHYLLOBOTHRIUM ERINACEI*

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Abstract: Mice infected with *Diphyllobothrium erinacei* plerocercoids were administered orally with paromomycin sulphate, bithionol, mebendazole and flubendazole at the total doses of 10,000 mg/kg for 200 days or subcutaneously with the drugs at the total doses of 2,500 mg/kg for 25 days. In spite of the high doses, no difference was observed between the number and weight of larvae recovered from mice in experimental groups and those in control groups.

Human cases infected with plerocercoids of *Diphyllobothrium erinacei* have been found one after another in Japan (Mukai *et al.*, 1977; Sasaki *et al.*, 1980; Kusunoki *et al.*, 1980; Mukai *et al.*, 1981). As reviewed in these papers, some Japanese eat inadequately cooked flesh of snakes harbouring the plerocercoids for healthy life or in the hope of finding health. Human sparganosis thus resultant often involves such difficulties that it cannot be treated satisfactorily. In some cases surgical removal of the larvae in subcutaneous tissues successfully leads to rapid recovery. However, in other cases with visceral larvae, their discovery and treatment are very difficult even if immunological diagnosis is made. This is why establishment of suitable chemotherapy is required. According to Faust *et al.* (1970), injection of ethanol with procaine (free of epinephrine) into the lesion and intravenous administration of norvarsenobenzol are effective for the treatment of ocular sparganosis in Vietnam. However, no experimental studies except a preliminary study by Maki and Yanagisawa (1983) have been reported regarding chemotherapy of sparganosis to the present authors' knowledge. This communication describes the first trial of drugs in infected mice. The following drugs were examined with the background of previous reports. Paromomycin sulphate was reported to be effective against various tapeworms including *D. erinacei* adults (Kagei and Hayashi, 1979; Orima *et al.*, 1981; Suzuki *et al.*, 1982). Bithionol is effective in elimination of *D. erinacei* adults (Oshima, 1976; Yoshimura, 1976). Both mebendazole and flubendazole have been found to be effective against hydatids of *Echinococcus granulosus* and *E. multilocularis* in animals (Schantz *et al.*, 1982; Van den Bossche, 1982). According to the report of Heath *et al.* (1975), mebendazole has lethal effect on cysticerci of *Taenia pisiformis* and tetrathyridia of *Mesocestoides corti* as well as hydatids of *E. granulosus*. Furthermore,

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flubendazole was reported to be effective on cysticerci of *Taenia solium* in pigs (Telléz-Girón *et al.*, 1981).

The anterior parts (about 4 mm long) of plerocercoids of *D. erinacei* isolated from naturally infected snakes, *Elaphe quadrivirgata* and washed with saline containing 1,000 units of penicillin and 500 μg of streptomycin per m*l* were inoculated subcutaneously into female mice of ICR-strain, 4 weeks old at the centre of the back using a hypodermic syringe and a needle (10 head parts/mouse).

The infected mice were divided at random into experimental and control groups of 6-7 mice each. Paromomycin sulphate (potency: 715 μg/mg; kindly supplied by Kyowa Hakko Kogyo Ltd.) dissolved in 1% (v/v) Tween 80, and pure powdered bithionol (kindly supplied by Tanabe Pharmaceutical Co., Ltd.), mebendazole and flubendazole (pure powdered drugs of Janssen Pharmaceutica, Belgium provided by the courtesy of Dr. K. Okano, Animal Health Development, Fujisawa Pharmaceutical Co. Ltd.) suspended in 1% Tween 80 were given to the mice of experimental groups orally via a stomach tube or subcutaneously with a tuberculin needle and syringe (1 m*l*, 26 G × 1/2). The control mice were orally or subcutaneously given 1% Tween 80 alone. Medication was done as follows. In the first study, mice were administered with one of the 4 drugs orally at 100 mg/kg/day, every other day, 21-219 days post-inoculation. In the second study, mice were administered with one of the 3 drugs, paromomycin sulphate, bithionol and mebendazole at 100 mg/kg/day for 25 consecutive days (98-122 days post-infection), unless otherwise indicated, subcutaneously at the centre of the back. Thus 10,000 and 2,500 mg/kg of the drugs in total doses were given in the first and second study, respectively to all the mice of experimental groups except those which died before the termination of medication. The skin of mice killed after the medication or those which died during the medication period was removed to recover the larvae from various body regions including the thoracic and abdominal cavities. This autopsy was carried out on 234-235 or 125 days post-infection in the first and second experiment, respectively with the exception that the mice which died during the medication period were examined for the worm recovery within 24 h after the death of mice. All the larvae recovered from each mouse were found to be alive. They were immersed in 10% (v/v) formalin, washed in saline, dried at 100°C, 3 h and weighed to the nearest mg.

Oral administration of paromomycin sulphate, bithionol, mebendazole or flubendazole even in the total dose of 10,000 mg/kg had no effect on the plerocercoids in mice (Table 1).

There was the possibility that no efficacy as shown in Table 1 was due to very low concentrations of the drugs absorbed from the intestine of mice used. Therefore, subcutaneous administration of paromomycin sulphate, bithionol and mebendazole was carried out. However, no difference was seen between the number of worms recovered from medicated and non-medicated groups as shown in Table 2. Mean dry weight of worms recovered from mice given 2,500 mg/kg in total dose was similar to that from control mice (Table 2).

In view of the fact that fairly many mice did not survive the administration of high doses (Tables 1, 2), the total doses of 2,500 (subcutaneous administration) and 10,000 (oral administration) mg/kg are higher than tolerable doses. Nevertheless no efficacy was seen in the 4 drugs against *D. erinacei* plerocercoids in mice.

Paromomycin sulphate and bithionol were reported to be effective against adult *D. erinacei*, and the 2 benzimidazoles, mebendazole and flubendazole to be effective against a number of larval tapeworms (cyclophyllidea) in host tissues as mentioned above. The 4

Table 1 Plerocercoids recovered from mice orally given paromomycin sulphate, bithionol, flubendazole or mebendazole

Medication mg/kg/day×days	total doses	Number of plerocercoids recovered per mouse	[Mean±S.D. (No. mice)]	Dry weight of plerocercoids recovered per mouse (mg)	[Mean±S.D. (No. mice)]
Paromomycin sulphate					
100× 64	6,400*	8		N. M.	
100× 81	8,100*	9		N. M.	
100×100	10,000	8, 9, 9, 10, 10	[9.2±0.8(5)]	87, 88, 92, 107, 121	[98.9±14.6(5)]
Bithionol					
100× 45	4,500*	10, 10		N. M.	
100× 59	5,900*	7		N. M.	
100×100	10,000	9, 9, 10, 10	[9.5±0.6(4)]	91, 126, 132, 149	[124.5±24.4(4)]
Mebendazole					
100× 3	300*	6		N. M.	
100× 33	3,300*	10		N. M.	
100× 68	6,800*	7		N. M.	
100× 70	7,000*	7		N. M.	
100×100	10,000	7, 9	[8.0±1.4(2)]	104, 106	[105.0±1.4(2)]
Flubendazole					
100×100	10,000	8, 9, 9, 10, 10, 10, 10	[9.4±0.8(7)]	79, 85, 86, 104, 105, 107, 126	[99.0±16.4(7)]
Control	0	7, 8, 8, 9, 9, 10, 10	[8.7±1.1(7)]	48, 77, 84, 88, 98, 100, 105	[85.9±19.4(7)]

Female mice of ICR strain 4 weeks old were subcutaneously given 10 head parts of plerocercoids, orally administered with either of the drugs at 100 mg/kg/day, every other day, 21-219 days post-infection and autopsied on 234-235 days post-infection. *Mice died during the medication period, being autopsied within 24 h after death. N. M.=not measured.

drugs were not effective against larval *D. erinacei* in mouse tissues. The reason for this fact is an open question to be clarified.

Bithionol was administered to patients suffering from larval *D. erinacei* infections but no evidence indicating curative efficacy of the drug was obtained (Araki *et al.*, 1976; Fujiwara *et al.*, 1978). If the 4 drugs are not effective in human cases as in infected mice of the present studies, the drugs including bithionol should not be administered. Further studies are needed to find suitable drugs against sparganosis.

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Table 2 Plerocercoids recovered from mice subcutaneously given paromomycin sulphate, bithionol or mebendazole

Medication mg/kg/day×days	total doses	Number of plerocercoids recovered per mouse	[Mean±S.D. (No. mice)]	Dry weight of plerocercoids recovered per mouse (mg)	[Mean±S.D. (No. mice)]
Paromomycin sulphate					
100× 22	2,200*	7		N. M.	
100× 25	2,500*	7, 7, 10, 10, 10, 10	[9.0±1.5(6)]	63, 67, 91, 95, 105, 109	[88.3±19.2(6)]
500× 1	500*	8, 8, 9, 10	[8.8±1.0(4)]	N. M.	
500× 3	1,500*	8, 9	[8.5±0.7(2)]	N. M.	
500× 11	5,500*	7		N. M.	
Bithionol					
100× 13	1,300*	7		N. M.	
100× 15	1,500*	7		N. M.	
100× 22	2,200*	10		N. M.	
100× 24	2,400*	3		N. M.	
100× 25	2,500	7, 9	[8.0±1.4(2)]	55, 72	[63.5±12.0(2)]
Mebendazole					
100× 23	2,300*	7		N. M.	
100× 25	2,500	3, 9, 9, 10, 10	[8.2±2.9(5)]	32, 86, 91, 99, 126	[86.8±34.3(5)]
Control	0	10, 10, 10, 10, 10, 10, 10	[10.0±0(7)]	76, 78, 81, 85, 92, 98, 103	[87.6±10.3(7)]

Female mice of ICR strain 4 weeks old were subcutaneously given 10 head parts of plerocercoids, subcutaneously administered with either of the drugs at 100 (or 500) mg/kg/day for 25 consecutive days (98-122 days post-infection) and autopsied 125 days post-infection. *Mice died during the medication period, being autopsied within 24 h after death. N. M.=not measured.

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マンソン孤虫に対する硫酸パロモマイシン，ビチオノール，メベンダゾールおよびフルベンダゾールの駆虫効果に関する実験的研究

牧　　純・柳沢十四男

マンソン孤虫を感染させたマウスに硫酸パロモマイシン，ビチオノール，メベンダゾールまたはフルベンダゾールを総量10,000 mg/kg・200日間経口投与，或いは総量2,500mg/kg・25日間皮下投与した。しかし実験群と対照群の間に，回収した虫体の数，および重量について差が認められなかった。

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NOTES ON BLACKFLIES (DIPTERA: SIMULIIDAE) FROM MYANMAR (FORMERLY BURMA)

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Abstract: Adult pinned specimens of Burmese Simuliidae held in British Museum (Natural History) were examined. A total of eight taxa recognized are all assigned in the genus *Simulium* Latreille *s. l.* and are further placed in the following subgenera: *Nevermannia* Enderlein (3 species including 2 new species), *Gomphostilbia* Enderlein (1 new species), *Himalayum* Lewis (1 species) and *Simulium* Latreille *s. str.* (3 species). Descriptions and illustrations for 3 new species and 1 unnamed one are presented.

INTRODUCTION

The simuliid fauna of Myanmar (formarly Burma) has been poorly studied. No blackfly species has been so far reported except *Simulium* (*Himalayum*) *indicum* Becher, 1985 which was recorded from female specimens (Lewis, 1974). The early stages of this species have remained unknown from Myanmar.

This paper reports additional seven blackfly species including three new species, based on adult simuliid specimens loaned from British Museum (Natural History), London.

MATERIAL AND METHODS

All the specimens examined were collected in 1934 by R. Malaise from Kambaiti, and in 1938 by R. Kaulback from Nam Tamali Valley, in northern Myanmar.

Some pinned adult specimens were dissected under a stereoscopic microscope and their head and genitalia were observed in detail after being immersed in 10% KOH solution. They were finally mounted on a glass slide, together with certain other parts of the body.

The laboratory procedures used in this work were almost the same as those described previously (Takaoka, 1983). The measurement of the hind basitarsus and tarsal claw tooth follows that of Davies and Györkös (1987).

The morphological features and terms used follow those of Crosskey (1969). The classification of Crosskey (1981) is followed except one of the subgenera, i.e., *Montisimulium*, which is not adopted in this study. One new species apparently belonging to this subgenus is included in the subgenus *Nevermannia* Enderlein.

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SPECIES ACCOUNTS

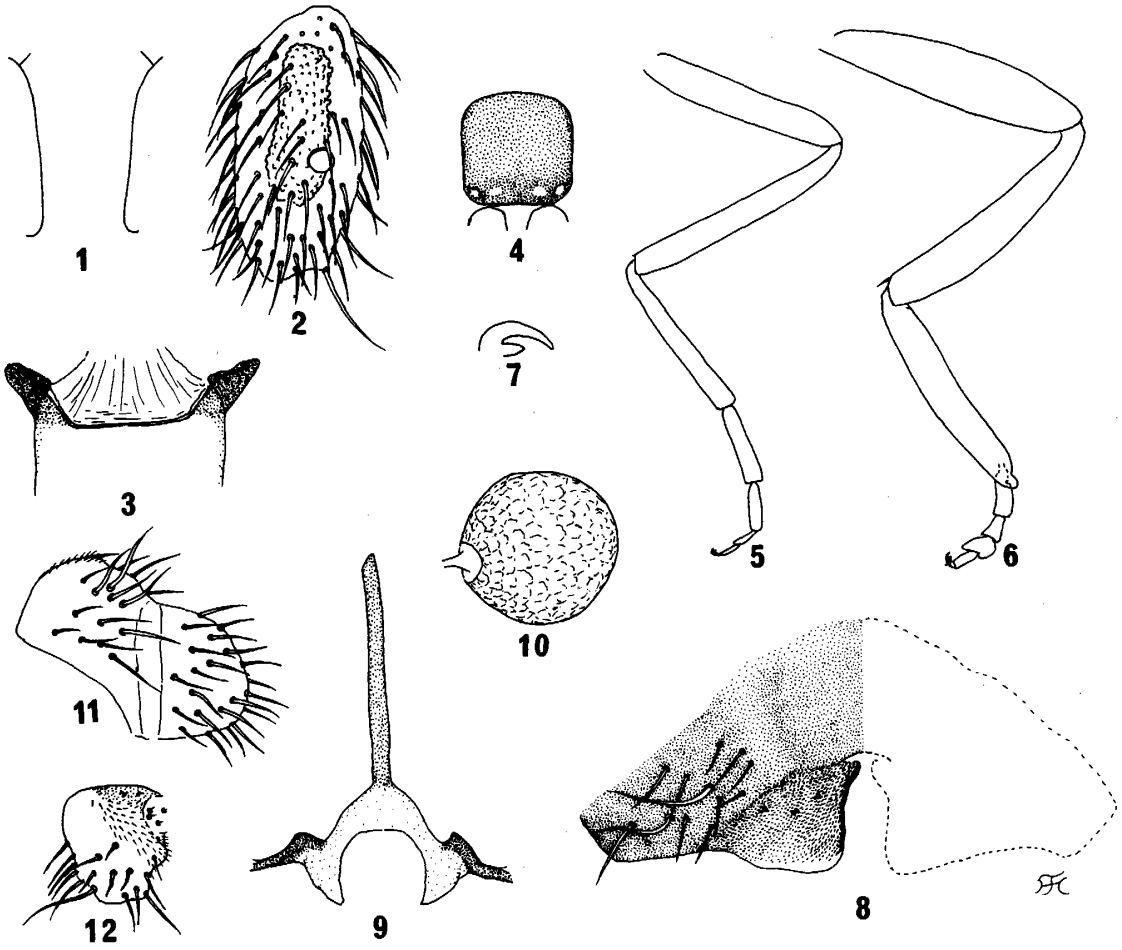
1. Simulium (Nevermannia) burmense sp. nov.

Female. Wing length 2.8 mm. *Head*. Frons (Fig. 1) and clypeus black, heavily white pruinose, and covered with numerous hairs. Frontal ratio 1.6:1.0:2.1. Frons-head ratio 1.0:4.8. Antenna composed of 2+9 segments, dark brown except scape and pedicel orange. Maxillary palp with 5 segments, proportion of apical 3rd, 4th and 5th segments 1.3:1.0:5.9; sensory vesicle (Fig. 2) elongated, ca. 3/5× length of 3rd segment. Maxilla with 15 strong teeth on each side. Mandible with ca. 40 small inner teeth and 13 outer teeth. Cibarium (Fig. 3) without any tubercles or teeth medially. *Thorax*. Scutum brownish black, entirely whitish grey pruinose when viewed in certain angle of direct light, or with 2 pairs of white pruinose spots each submedially and laterally (on prescutellar regions) near anterior margin when viewed in another angle (Fig. 4); scutum densely covered with recumbent golden pubescence, and without any longitudinal lines. Scutellum brown with numerous golden pubescence and several upstanding dark hairs. Postscutellum blackish brown, white pruinose and bare. Pleural membrane and katepisternum bare. *Legs*. All coxae and trochanters yellow except mid and hind coxae dark. All femora yellow except apical small portion dark brown. All tibiae dark brown with median large portion yellow to light brown; all femora and tibiae densely covered with golden hairs. All tarsi brown to dark brown. Fore basitarsus (Fig. 5) slender, cylindrical, ca. 7.5× as long as its greatest width. Hind basitarsus nearly parallel sided (Fig. 6). Calcipala and pedisulcus moderately developed. Claw with basal tooth, ca. 1/2× length of claw (Fig. 7). *Wing*. Costa with parallel rows of short spinules as well as hairs. Subcosta with hairs. Basal section of radius fully haired. Hairs at base of stem vein dark. *Abdomen*. Basal scale pale yellow, with golden fringe. Dorsal surface of abdomen dark brown, with minute hairs, and somewhat shiny on segments 7-9. *Genitalia*. Sternite 7 large and well demarcated. Sternite 8 bare medially and with ca. 12 long hairs on each side (Fig. 8). Anterior gonapophyses simple, nearly triangular, and membraneous with a few microsetae; inner margin slightly curved and narrowly sclerotized; posterior border thin and transparent. Genital fork with wide arms; stem and anterolateral margin of arms heavily sclerotized; arms expanded posteromedially (Fig. 9). Spermatheca almost globular in shape, strongly sclerotized and with reticulate pattern (Fig. 10). Paraproct short, somewhat produced ventrally and moderately haired (Figs. 11 and 12). Cercus semicircular in side view, ca. 3/5× as long as its width at base (Fig. 11).

Male, pupa and larva. Unknown.

Type specimens. Holotype female, slide-mounted except thorax, left fore and mid legs and right wing left on pin. MYANMAR: Kambaiti, 7,000 ft., 30. IV. 1934, R. Malaise. Paratype female, pinned except terminal tip of abdomen on slide, same data as holotype.

Remarks. This new species is assigned to the *vernum* group of the subgenus *Nevermannia* defined by Crosskey and Davies (1972), and shows similarities to *S. gracilis* and *S. puri*, both described from India by Datta (1973). *Simulium burmense* sp. nov. differs from the former species by the shape of the posterior portion of cibarium, and from the latter by the shape of the genital fork.



Figs. 1-12 *Simulium (Nevermannia) burmense* sp. nov. female. 1, frons; 2, 3rd segment of maxillary palp showing sensory vesicle; 3, cibarium; 4, scutum showing two pairs of whitish spots; 5, fore leg (coxa and trochanter omitted); 6, hind leg (coxa and trochanter omitted); 7, claw; 8, 8th sternite and anterior gonapophyses; 9, genital fork; 10, spermatheca; 11, paraproct and cercus in lateral view; 12, paraproct in ventral view.

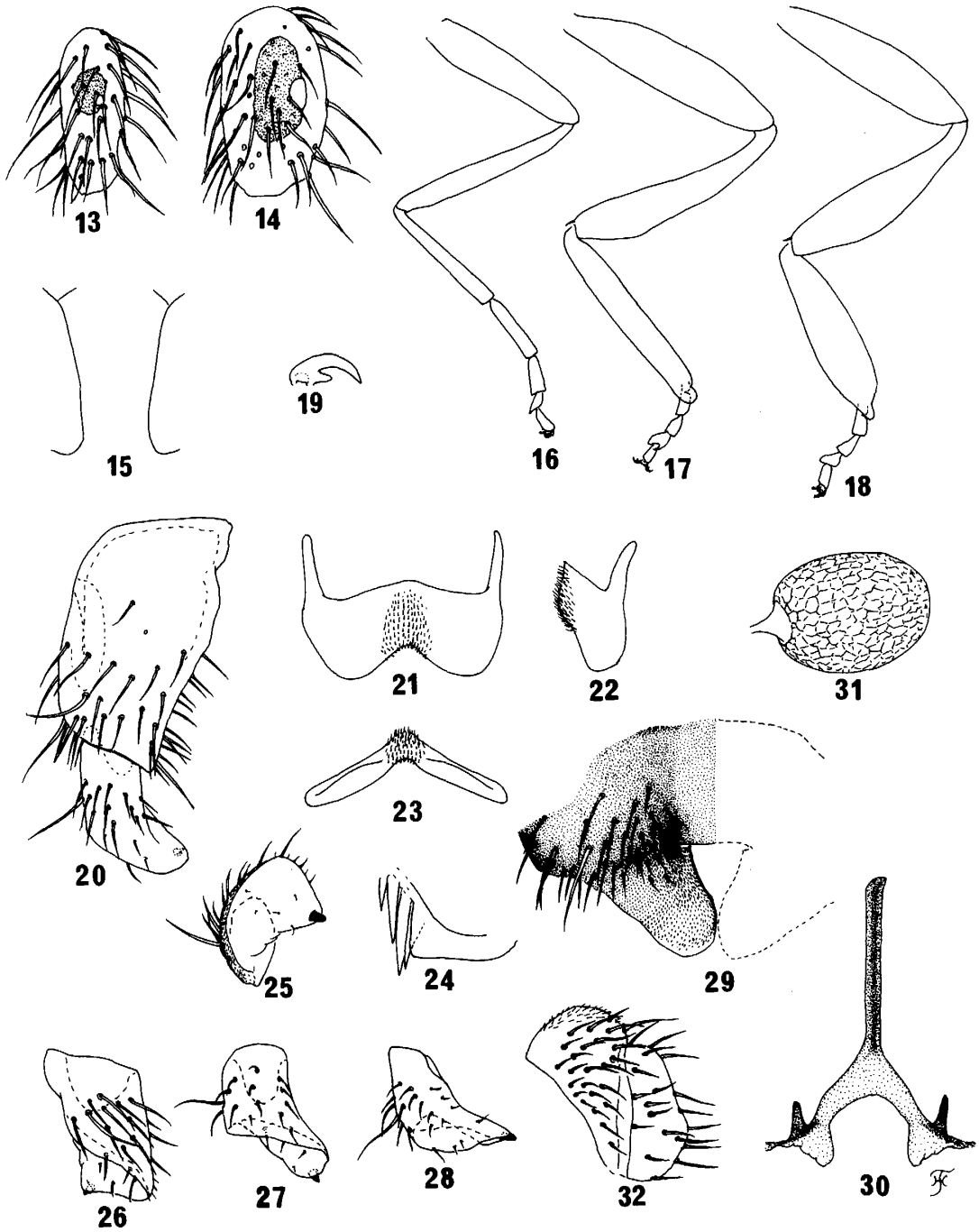
This species is also related to *S. aberrans* Delfinado, 1969 from Philippines (Takaoka, 1983) and *S. taulingense* Takaoka, 1979 from Taiwan (Takaoka, 1979). However, *S. aberrans* differs by the female antenna and legs entirely dark brown, and *S. taulingense* shows the different shape of the cercus (triangular in side view).

2. Simulium (Nevermannia) kambaitense sp. nov.

Male. Wing length 2.8 mm. *Head.* slightly wider than thorax. Upper eye consisting of 19 horizontal rows and 19 vertical columns of somewhat enlarged facets. Clypeus brownish black, whitish grey pruinose, and with dark hairs. Antenna composed of 2+9 segments, dark brown except base of 1st flagellar segment pale; 1st flagellar segment elongate, ca. 2× as long as 2nd flagellar segment. Maxillary palp with 5 segments, with 3rd, 4th and 5th segments in proportional length of 1.0:1.3:2.3; sensory vesicle small, ca. 1/4× as long as 3rd segment (Fig.

13). *Thorax.* Scutum brownish black, whitish grey pruinose, densely covered with golden recumbent pubescence. Scutellum brown with numerous golden hairs and several dark hairs. Postscutellum brownish black, whitish grey pruinose and bare. Pleural membrane and katepisternum bare. *Legs.* All coxae and trochanters dark yellow except hind coxa brown. All femora dark yellow to pale brown except apical portion dark brown. All tibiae dark brown with median large portion somewhat pale. All tarsi dark brown except hind basitarsus and basal 1/2 of 2nd tarsal segment pale brown. Fore basitarsus slender, cylindrical, ca. 10× as long as its greatest width. Hind basitarsus enlarged, spindle-shaped, its greatest width almost the same as the greatest width of hind femur and tibia (Fig. 18). Calcipala and pedisulcus well developed. *Wing.* Costa with 2 parallel rows of short spines as well as dark hairs. Subcosta bare. Basal portion of radius fully haired. Basal tuft hairs dark brown. *Abdomen.* Basal scale brown with golden hair fringe. Dorsal surface of abdomen brownish black with golden and dark short hairs. *Genitalia.* Coxite enlarged, much longer than wide (Fig. 20). Style shorter than coxite, twisted dorso-inwardly, and with a single apical spine directed dorso-inwards (Figs. 25-28); posterodorsal surface thin, membraneous and smooth except a few minute setae (Fig. 25). Ventral plate lamellate, well sclerotized, much wider than long, and covered ventrally and posteriorly with minute setae in the middle (Fig. 21); ventral surface somewhat raised ventrally in the center (Fig. 23), and posterior border deeply concave (Fig. 21). Paramere with 4 stout spines (Fig. 24). Median sclerite simple, rod-like.

Female. Wing length 2.8 mm. *Head.* Frons (Fig. 15) and clypeus brownish black, thickly whitish grey pruinose, and densely covered with golden pubescence, intermixed with sparse dark hairs. Frotal ratio 1.7:1.0:2.3. Frons head ratio 1.0:4.8. Antenna composed of 2+9 segments, and dark brown except scape and pedicel greyish yellow. Maxillary palp composed of 5 segments, with 3rd, 4th and 5th segments in proportional length of 1.0:1.0:1.5; 3rd segment somewhat enlarged, with elongated sensory vesicle (Fig. 14), slightly over 1/2× as long as 3rd segment. Maxilla with 10 or 11 inner teeth and 14 or 15 outer ones. Mandible with ca. 28 inner teeth and without any outer teeth. Cibarium without any denticles. *Thorax.* Scutum brownish black, thickly whitish grey pruinose, and densely covered with golden recumbent pubescence; no longitudinal lines discernible. Scutellum brown with golden pubescence and several erect dark hairs. Postscutellum brownish black, whitish grey pruinose, and bare. Pleural membrane and katepisternum bare. *Legs.* All coxae, trochanters and femora light yellow except mid and hind coxae, and apical tip of all femora brown. All tibiae and tarsi brown to dark brown except middle large portion of tibiae dark yellow to light brown. All femora and tibiae densely covered with golden hairs. Fore basitarsus (Fig. 16) slender, cylindrical, ca. 7.4× as long as its greatest width. Hind basitarsus (Fig. 17) parallel-sided. Calcipala and pedisulcus well developed (Fig. 17). Claw with medium basal tooth, ca. 0.37× length of claw (Fig. 19). *Wing.* Costa with 2 parallel rows of short spines as well as hairs. Subcosta fully haired. Basal portion of radius fully haired. Basal hair tuft dark brown. *Abdomen.* Basal scale dark yellow with golden hair fringe. Dorsal surface of abdomen dark brown, with terga somewhat grey pruinose, and with light and dark short hairs; terga 7-9 shiny when viewd in light. *Genitalia.* Sternite of 7th abdominal segment widely developed. Sternite 8 (Fig. 29) bare medially but furnished with ca. 36 stout hairs on each side. Anterior gonapophyses (Fig. 29) thin, membraneous, produced posteromedially,



Figs. 13-32 *Simulium* (*Nevermannia*) *kambaitense* sp. nov. male and female. 13 and 14, 3rd segments of maxillary palp (13 for male, 14 for female); 15, frons of female; 16, fore leg of female; 17, hind leg of female; 18, hind leg of male (coxa and trochanter omitted in Figs. 16-18); 19, claw of female; 20, coxite and style in ventral view; 21, ventral plate in ventral view; 22, ventral plate in side view; 23, ventral plate in end view; 24, paramere with 4 hooks; 25, style in end view; 26, style in outside view; 27, style in ventolateral view; 28, style in inside view; 29, 8th sternite and anterior gonapophyses; 30, genital fork; 31, spermatheca; 32, paraproct and cercus in lateral view.

appearing tongue-like, narrowly sclerotized on inner border, bare and transparent near rounded posteromedial border; a few short setae near anterior border which is not well demarcated. Genital fork (Fig. 30. with well sclerotized stem and wide arms; arm with stout, long projection directed forwards, as well as rounded projection directed posteromedially. Spermatheca (Fig. 31) ovoid in shape, strongly sclerotized and with reticulate pattern. Paraproct (Fig. 32) short, not produced under cercus, and moderately setose. Cercus (Fig. 32) short, ca. 2/5× as long as its width, and moderately setose.

Type specimens. Holotype male, slide-mounted, MYANMAR: Kambaiti, 7,000 ft., 30. IV. 1934, R. Malaïse. Allotype female, slide-mounted, same data as holotype. Paratypes 3 females, slide-mounted, same data as holotype; 1 male, pinned (genitalia on slide), 1 female, pinned, 5.V.1934, same locality as holotype.

Remarks. Simulium kambaitense sp. nov. seems to belong to the *montium* group of the subgenus *Nevermannia* (Rubtsov, 1959-64) (i.e., subgenus *Montisimulium* in Crosskey, 1981) by the shape of the genitalia of both sexes.

The male of this species is similar to that of *S. ghoomense* Datta, 1975 from India (Datta, 1975) but differs from the latter by the shape of the style. This species is also similar to the other Indian related species, *S. nemorivagum* Datta, 1973 (Datta, 1973), from which this is readily separated by the ventral plate with a depressed posterior border.

The morphological characters of the female (herein described as a probable female of *S. kambaitense*) resemble those of *S. nemorivagum* (Datta, 1974) and *S. chowi* Takaoka, 1979 from Taiwan (Takaoka, 1979). There are slight differences in the shape of the genital fork between these known species and the present new species. The female genitalia (e.g., the shapes of anterior gonapophyses and genital fork) of *S. ghoomense* illustrated by Datta (1975) is different from those of this new species.

3. Simulium (Nevermannia) sp. A.

Female. Wing length 3.1 mm. *Head.* Frons and clypeus brownish black, thickly whitish grey pruinose, and densely covered with golden pubescence, intermixed with sparse dark hairs. Frontal ratio 2.2:1.0:3.4 (this ratio was variable, being 2.0:1.0:2.7 and 1.7:1.0:2.7 in 2 other specimens examined). Frons head ratio 1.0:5.1 (also variable, i.e., 1.0:4.5 and 1.0:5.7 in 2 other specimens). Antenna composed of 2+9 segments, and dark brown except scape and pedicel yellow. Maxillary palp composed of 5 segments, with 3rd, 4th and 5th segments in proportional length of 1.3:1.0:1.9; 3rd segment somewhat enlarged, with elongated sensory vesicle, ca. 2× as long as its width, and slightly over 1/2× length of 3rd segment. Maxilla with 9 inner teeth and 13 outer ones. Mandible with ca. 20 inner teeth and without any outer teeth. Cibarium without any denticles. *Thorax.* Scutum reddish brown, whitish grey pruinose, with 3 longitudinal dark lines, and densely covered with golden recumbent pubescence. Scutellum light brown, whitish grey pruinose and with golden pubescence and several erect dark hairs. Postscutellum brown, whitish grey pruinose, and bare. Pleural membrane and katepisternum bare. *Legs.* All coxae, trochanters and femora light to dark yellow except mid and hind coxae, and apical tip of all femora light brown. All tibiae brown except middle large portion dark yellow to light brown. All tarsi dark brown except most of hind basitarsus and basal 1/2 of hind 2nd tarsal segment light brown. All femora and tibiae densely covered with

golden hairs. Fore basitarsus slender, cylindrical, ca. 8.5× as long as its greatest width. Hind basitarsus (Fig. 33) parallel-sided. Calcipala and pedisulcus well developed (Fig. 33). Claw with large basal tooth, ca. 0.4× length of claw (Fig. 34). *Wing.* Costa with 2 parallel rows of short spines as well as hairs. Subcosta fully haired. Basal portion of radius fully haired. Basal hair tuft dark brown. *Abdomen.* Basal scale light brown with a fringe of golden hairs. Dorsal surface of 2nd abdominal segment pale and somewhat pruinose in light; rest of abdominal segments brown with golden and dark short hairs; terga 7-9 semishiny. *Genitalia.* Sternite of 7th abdominal segment widely developed. Sternite 8 (Fig. 35) bare medially but furnished with ca. 16 stout hairs on each side. Anterior gonapophyses (Fig. 35) thin, membraneous, triangular in shape, with a few short setae; inner border narrowly sclerotized, and posteromedian corner rounded, transparent and bare. Genital fork (Fig. 36) with well sclerotized stem and wide arms; arm with stout, long projection directed forwards, as well as rounded projection directed posteromedially. Spermatheca (Fig. 37) ovoid in shape, strongly sclerotized and with reticulate pattern. Paraproct (Fig. 38) short, not produced under cercus, and moderately setose. Cercus (Fig. 38) short, ca. 1/2× as long as its width, and moderately setose.

Specimens examined. 1 female, pinned, MYANMAR: Kambaiti, 7,000 ft., 30. IV. 1934, R. Malaise, 3 females, slide-mounted, same data as first specimen except collection date of 2 females, i.e., 2.V.1934 and 5.V.1934.

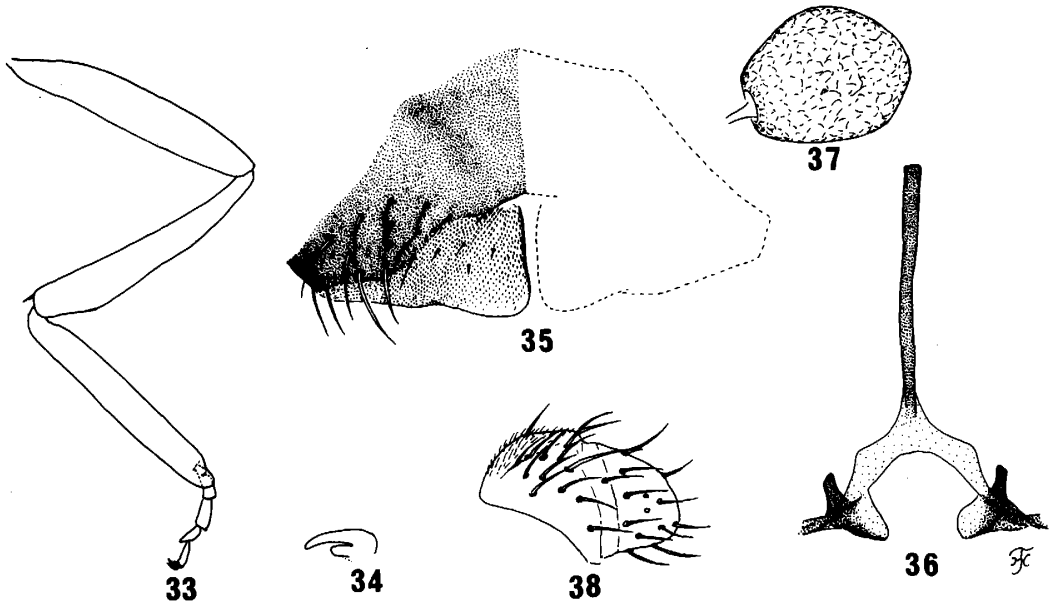
Remarks. This species belongs to the *feuerborni* group of the subgenus *Nevermannia*, as defined by Datta (1973), to which several species are assigned. The female of this species is very similar to those of *S. praelargum* Datta, 1973 from India (Datta, 1973), and *S. chitoense* Takaoka, 1979 from Taiwan (Takaoka, 1979) in many features including the slender fore basitarsus (width-length ratio, 1:8.5), but is distinguished from these two species by the number of macrosetae on the eighth sternite (ca. 16 versus over 20 on each side). The characters of the fore basitarsus and genitalia of this species agree with those of *S. perulucidulum* Takaoka, 1983 from Philippines (Takaoka, 1983), but there are differences in the number of teeth of the maxilla between the two species.

Despite these differences, it would be better to leave this species unnamed because it is impossible to compare with other related species, e.g., *S. senile* Brunetti, 1911 from India (Brunetti, 1911), *S. fuscinervis* Edwards, 1933 from Borneo (Edwards, 1933), *S. bryopodium* Delfinado, 1971 from Palawan (Delfinado, 1971), and *S. feuerborni* Edwards, 1934 from Java and Bali (Edwards, 1934) because the female of these species was still unknown. The pupa and larva of an unnamed species of the same species group have been reported from Thailand as *S.* (*Eusimulium*) sp. B by Takaoka and Suzuki (1984). Future studies may solve whether the present specimens represent a new species or are the female of one of the related species mentioned above.

There is a possibility that the female specimens examined consist of more than one species because a frontal ratio of these specimens varied remarkably as noted above, although the character of the genitalia was almost the same.

4. Simulium (Gomphostilbia) namense sp. nov.

Male. Wing length 2.5 mm. *Head.* slightly wider than thorax. Upper eye consisting of 15



Figs. 33-38 *Simulium* (*Nevermannia*) sp. A female. 33, hind leg (coxa and trochanter omitted); 34, claw; 35, 8th sternite and anterior gonapophyses; 36, genital fork; 37, spermatheca; 38, paraproct and cercus in lateral view.

horizontal rows and 14 vertical columns of enlarged facets. Clypeus brownish black, whitish grey pruinose, and with dark hairs. Antenna composed of 2+9 segments, dark brown except base of 1st flagellar segment pale; 1st flagellar segment elongate, ca. 2× as long as 2nd flagellar segment. Maxillary palp with 5 segments, with 3rd, 4th and 5th segments in proportional length of 1.0:1.2:2.7; sensory vesicle small, ca. 1/5.5× as long as 3rd segment (Fig. 39). *Thorax.* Scutum dark brown, whitish grey pruinose, with 3 faint dark longitudinal lines, and densely covered with golden recumbent pubescence. Scutellum brown, whitish grey pruinose, with numerous golden hairs and several dark hairs. Postscutellum brownish black, whitish grey pruinose and bare. Pleural membrane bare. Katepisternum haired. *Legs.* All coxae dark brown except fore coxa whitish yellow. All trochanters whitish yellow. All femora brown except apical portion dark brown. Fore tibia dark brown with median large portion somewhat whitish yellow. Mid and hind tibiae whitish on basal 2/5 and dark brown on apical 3/5. All tarsi dark brown except basal 1/4 of mid basitarsus somewhat pale and basal 2/5 of hind basitarsus whitish. Fore basitarsus slender, cylindrical, ca. 9× as long as its greatest width. Hind basitarsus inflated distally, wedge shaped, ca. 3.3× as long as its greatest width; greatest width almost the same as the greatest width of hind tibia but slightly wider than hind femur (Fig. 42). Calcipala and pedisulcus well developed. *Wing.* Costa with 2 parallel rows of short spines as well as dark hairs. Subcosta bare or with a few hairs. Basal portion of radius fully haired. Basal tuft hairs golden yellow. *Abdomen.* Basal scale dark brown with greyish yellow hair fringe. Dorsal surface of abdomen dark brown with dark hairs; terga 2,5,6 and 7 dorsolaterally grey pruinose and shiny. *Genitalia.* Coxite enlarged, ca 1.8× as long as its width (Fig. 47). Style shorter than coxite, gently curved inwards and with a single spine (Fig. 47). Ventral plate lamellate, well sclerotized, much wider than long, and covered ventrally, posteriorly and dorsally with minute setae (Fig. 48); ventral surface

somewhat raised ventrally in the center (Fig. 49). Paramere with 4 stout spines, though innermost spine short (Fig. 51). Median sclerite simple, wide plate-like.

Female. Wing length 2.0 mm. *Head.* Frons and clypeus brownish black, thickly whitish grey pruinose, and densely covered with whitish yellow pubescence, intermixed with sparse dark hairs. Frontal ratio 1.9:1.0:2.6. Frons head ratio 1.0:4.9. Antenna composed of 2+9 segments, and dark brown except scape, pedicel and basal 1/2 of 1st flagellar segment greyish yellow. Maxillary palp composed of 5 segments, with 3rd, 4th and 5th segments in proportional length of 1.0:1.1:2.4; 3rd segment normal, with small, ovoid sensory vesicle (Fig. 40), ca. 1/4× as long as 3rd segment. Maxilla with 11 or 12 inner teeth and 14 outer ones. Mandible (Fig. 41) with ca. 26 inner teeth and with 3 or 4 outer teeth. Cibarium without any denticles. *Thorax.* Scutum brownish black, thickly whitish grey pruinose, with faint 3 longitudinal lines, and densely covered with golden recumbent pubescence. Scutellum brown with golden pubescence and several erect dark hairs. Postscutellum brownish black, whitish grey pruinose, and bare. Pleural membrane bare. Katepisternum haired. *Legs.* All coxae and trochanters whitish yellow except mid and hind coxae dark brown. Fore femur yellow on base, becoming dark toward apex. Mid femur brown except basal 1/4 yellow. Hind femur light brown with apical tip dark brown. Fore tibia whitish with apical 2/7 dark brown. Mid tibia whitish on basal 1/3, becoming dark towards apex, and dark brown on apical 1/3. Hind tibia whitish with apical 1/4 dark brown; its border not well defined. All femora and tibiae densely covered with whitish hairs. Fore tarsi dark brown. Mid and hind tarsi dark brown except basal 2/5 of mid basitarsus and basal 2/3 of hind basitarsus and basal 1/2 of hind 2nd segment whitish. Fore basitarsus (Fig. 43) slender, cylindrical, ca. 6.7× as long as its greatest width. Hind basitarsus (Fig. 45) parallel-sided. Calcipala and pedisulcus well developed (Fig. 45). Claw with large basal tooth, ca. 1/2× length of claw (Fig. 46). *Wing.* Costa with 2 parallel rows of short spines as well as hairs. Subcosta fully haired. Basal portion of radius fully haired. Basal hair tuft golden yellow. *Abdomen.* Basal scale greyish yellow with golden hair fringe. Dorsal surface of abdomen dark brown, with dark hairs; when viewed in light, tergum 2 somewhat grey pruinose and terga 7-9 shiny. *Genitalia.* Sternite of 7th abdominal segment undeveloped. Sternite 8 (Fig. 52) bare medially but furnished with ca. 20 stout hairs on each side. Anterior gonapophyses (Fig. 52) thin, membraneous, triangular in shape, narrowly sclerotized on inner border, bare and transparent near rounded posteromedial border; a few short setae near anterior border. Genital fork (Fig. 53) with well sclerotized stem and lacking any projections on its arms. Spermatheca (Fig. 54) ellipsoidal in shape, strongly sclerotized but without definite reticulate pattern. Paraproct (Figs. 55 and 56) short, not produced under cercus, and moderately setose. Cercus (Fig. 56) short, ca. 2/5× as long as its width, and moderately setose.

Type specimens. Holotype male, slide-mounted, MYANMAR: Kambaiti, 7,000 ft., 30. IV. 1934, R. Malaise. Allotype female, slide-mounted, 2.V. 1934, same locality as holotype. Paratypes 1 male, slide-mounted, same data as holotype, 1 female, pinned and 1 female, slide-mounted, both same data as allotype; 1 female, slide-mounted and 2 females, pinned, Nam Tamali, 3,000 ft., 8. VIII. 1938, R. Kaulback (BM 1938-741).

Remarks. The male of *S. namense* sp. nov. is characterised by the inflated wedge-shaped hind

basitarsus (Fig. 42). This character is shared by several *Gomphostilbia* species, most of which have been studied in detail by Davies and Györkös (1987) while they described *S. ela* from Sri Lanka.

This new species seems to be similar to *S. metatarsale* Brunetti, 1911 described from a single male specimen from Kurseong, India (Brunetti, 1911), from which this is distinguishable in the coloration of the fore femur (*metatarsale* in parenthesis): brown with apex dark brown (dull yellowish).

The male of *S. namense* shows similarities to that of *S.metatarsale* var. described by Edwards (1934) from Java in the coloration of the scutal hairs, hairs at the base of stem vein and legs, but differs from the latter by the size of hind basitarsus: width-length ratio 1:3.3 (1: 2.7 in *metatarsale* var. according to Davies and Györkös, 1987). The male of this new species is much more similar to the specimens of "*S. metatarsale*" reported from Taiwan (Takaoka, 1979). There are slight differences in the following characters (parentheses in *metatarsale*): number of vertical and horizontal rows of large facets 14 and 15 (10 or 11 and 13); color of antenna dark brown except base of the first flagellar segment pale (scape, pedicel and base of the first flagellar segment pale, and the rest dark brown). The female of *S. namense* also has a close similarity to that of the latter species but is differentiated by the very small sensory vesicle, which is ovoid and about 0.25× as long as third segment of the maxillary palp, whereas sensory vesicle is oblong and about 0.36× length of third segment in Taiwanese *metatarsale*.

Simulium namense is similar to *S. nepalense* from Nepal (Lewis, 1964) and *S. tenuistylum* from India (Datta, 1973), from which it differs also by the small sensory vesicle in the female and by the shape of the ventral plate in the male.

Three Japanese species, i.e., *S. ogatai* Rubtsov, 1962 (Rubtsov, 1959-1964), *S. tokarense* Takaoka, 1973 (Takaoka, 1973) and *S. okinawense* Takaoka, 1976 (Takaoka, 1976), share many characters with the present new species. The male of *S. namense* is separated from these three species by the dark antenna and also from *S. ogatai* by the smaller number of vertical and horizontal rows of large facets (17 rows in *ogatai*). There are also slight differences in the size of the female sensory vesicle between this new species and three other species (relative length of sensory vesicle against third segment of maxillary palp 0.25 in *namense* whereas 0.33 or more in others).

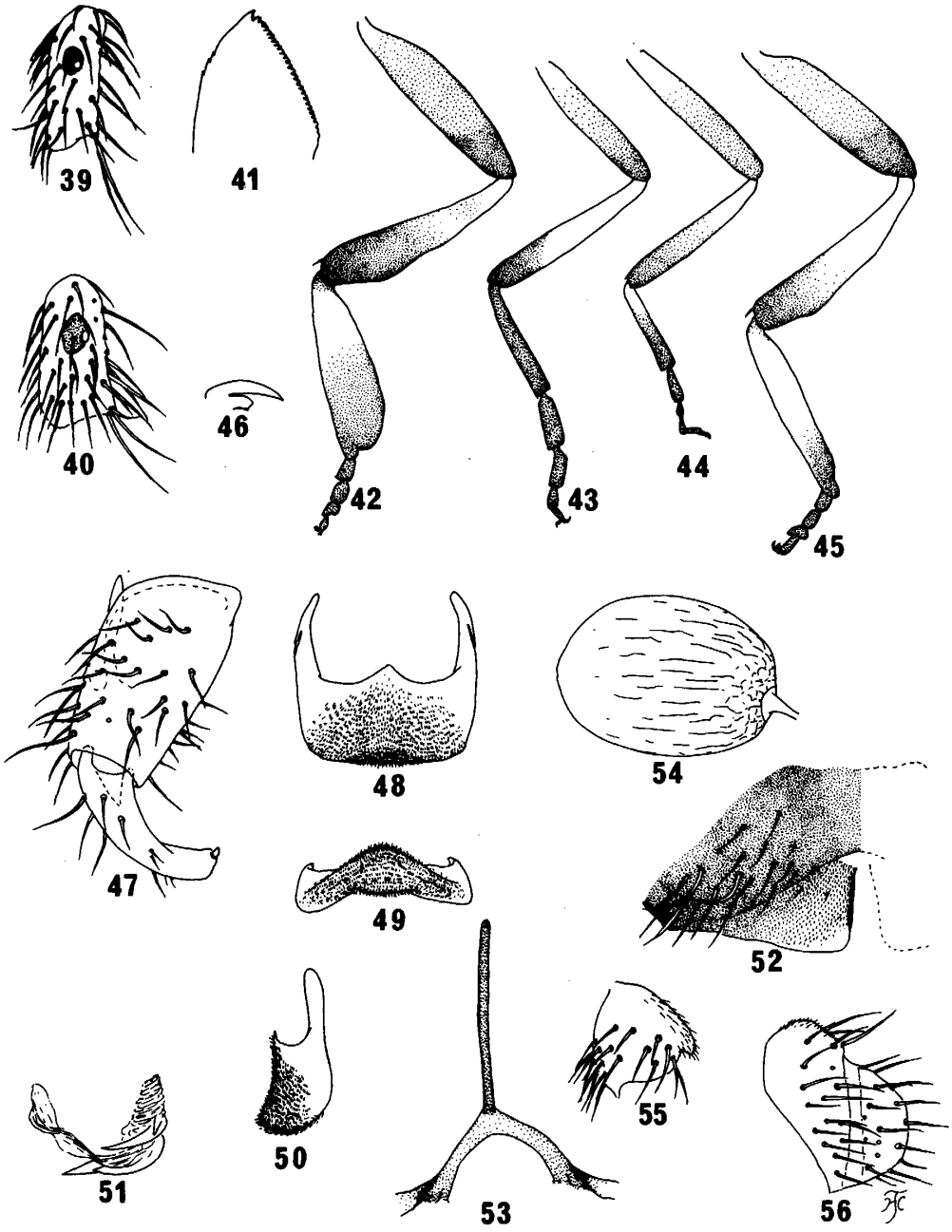
Simulium sp. C reported from Thailand (Takaoka and Suzuki, 1984) has the similar wedge-shaped hind basitarsus. The number of vertical and horizontal rows of large facets of this species is different from *S. namense* (i.e., 11 and 13 versus 14 and 15).

Simulium ela from Sri Lanka (Davies and Györkös, 1987) is easily distinguished from the new species by the brassy scutal hairs and dark hairs at the base of stem vein in both sexes, and by the large sensory vesicle, basal 1/3 of the hind tibia pale and the small number of stout hairs on the sternite 8 in the female.

This new species is distinguished from *S. rosemaryae* Takaoka et Roberts, 1988 from Sulawesi (Takaoka and Roberts, 1988) by the small sensory vesicle and the number of teeth on outer margin of the mandible in the female, and by the number of vertical columns of large facets in the male.

5. Simulium (Himalayum) indicum Becher, 1885

Simulium indicum Becher, 1885: 199-200.



Figs. 39-56 *Simulium* (*Gomphostilbia*) *namense* sp. nov. male and female. 39 and 40, male and female 3rd segment of maxillary palp, respectively; 41, apical tip of female mandible; 42, hind leg of male; 43-45, fore, mid and hind legs of female, respectively (coxae and trochanters omitted); 46, female claw; 47, coxite and style in ventral view; 48, ventral plate in ventral view; 49, ventral plate in end view; 50, ventral plate in side view; 51, paramere with 4 hooks; 52, 8th sternite and anterior gonapophyses; 53, genital fork; 54, spermatheca; 55, paraproct in ventral view; 56, paraproct and cercus in side view.

Simulium (*Himalayum*) *indicum*: Lewis, 1973: 462-463; Lewis, 1974: 25-33.

Material examined. 1 female, pinned except head mounted on glass slide, MYANMAR: Kambaiti, 7,000 ft., R. Malaise, 30. IV. 1934.

Distribution. Pakistan, India, Nepal, Bhutan, Myanmar, Bangladesh, China, Thailand.

Remarks. This species has been reported to be widely distributed in the Himalayas from Assam to Kashimir (Brunetti, 1911; Lewis, 1974; Datta, 1983). It should be remembered that considering its wide range of distribution, *S. indicum* may be a species complex consisting of more than one species, as already suggested by Takaoka and Suzuki (1984). The pupal and larval specimens are needed for final identification although the female specimen examined agrees with the description of *S. indicum* given by Lewis (1974). The female specimen examined is also similar to *S. nigrogilvum* Summers from Thailand (Takaoka and Suzuki, 1984) but the shape of the cibarial projection seems to be slightly different from each other.

6. Simulium (Simulium) chamlongi Takaoka et Suzuki, 1984

Simulium (*Simulium*) *chamlongi* Takaoka et Suzuki, 1984: 27-30.

Material examined. 1 pinned female, MYANMAR: Kambaiti, 7,000 ft., 30. IV. 1934, R. Malaise.

Distribution. Thailand, Myanmar (new record).

Remarks. The female specimen was provisionally identified as above because it almost agrees with the description of *S. chamlongi* described from Thailand (Takaoka and Suzuki, 1984). Further material is required for final identification. This is a first record of the *variegatum* species group from Myanmar.

7. Simulium (Simulium) novolineatum Puri, 1933

Simulium (*Simulium*) *novolineatum* Puri, 1933: 817 (replacement name for *S.* (*S.*) *lineatum* Puri, 1932b: 1125-30)

Material examined. 2 females, pinned except genitalia of 1 female mounted on glass slide, MYANMAR: Kambaiti, 7,000 ft., 30. IV. 1934, R. Malaise; 1 female, pinned, same data as 2 other specimens except 5.V. Myanmar 1934.

Distribution. India, Myanmar (new record).

Remarks. The genitalia of one female specimen examined conformed to those of *S. novolineatum* Puri, 1933 and *S. barraudi* Puri, 1932, both described from India (Puri, 1932b). The identification of these female specimens as *S. novolineatum* was based on their coloration of the legs, i.e., basal 1/2 of the mid basitarsus yellow (not basal 3/4 as in *barraudi*).

Puri (1932b) indicated that the coloration of mid and hind femora of the female was variable by localities. The present Burmese specimens, which have all the femora (even fore

femur) almost entirely dark, are close to the specimens of *S. novolineatum* from Mercara but are different from those from Marianbarie, Bengal Terai (type locality of this species), which show the femora yellowish except apical tip dark (Puri, 1932b).

This represents a first record of this species from Myanmar.

8. Simulium (Simulium) rufibasis Brunetti, 1911

Simulium rufibasis Brunetti, 1911: 282-88; Rubtsov, 1959-1964: 554.
Simulium (*Simulium*) *rufibasis*: Puri, 1932a: 899-903; Ogata *et. al.*, 1956: 94-95; Crosskey, 1973: 428; Datta, 1974a: 19-20; Takaoka, 1977: 213-216; Takaoka, 1979: 395; Takaoka and Suzuki, 1984: 41-42.

Material examined. 23 pinned females, 3 pinned males, and 1 male, slide-mounted, MYANMAR: Kambaiti, 7,000 ft., R. Malaise, 30. IV. 1934; 1 pinned female, same data as others except 2.V.1934; 2 pinned males, same data as others except 5.V. 1934.

Distribution. Pakistan, India, Thailand, Myanmar (new record), Taiwan, Japan.

Remarks. The female specimens almost agree with the description of *S. rufibasis* Brunetti, 1911 from India (Puri, 1932a). The female of this species has been reported to be identical to that of *S. ramosum* Puri, 1932 also from India, although these two species are distinguished in the male and pupal stage (Puri, 1932a). It is therefore possible that some or all of these female specimens are *S. ramosum*.

Three male specimens examined agree with the description of *S. rufibasis* given by Puri (1932a) in which the coloration of the male hind basitarsus is pale on basal 1/3 (not on basal 1/2 as *S. ramosum*).

This species is for the first time recorded from Myanmar.

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ミャンマー（ビルマ）のブユについて

高岡　宏行

大英自然史博物館に保管されていた，ミャンマー産ブユの成虫標本を，分類学的に検討した。ミャンマーからは，これまで1種しか報告がなかったが，今回の研究の結果，さらに7種の分布が明らかになった。本論文では，このうち新種と思われる3種の記載を行った。

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EFFECTS OF ENVIRONMENTAL TEMPERATURE AND MEMBRANE FEEDING SOLUTIONS OF PURIFIED OOKINETES ON THE SPOROGONIC DEVELOPMENT OF *PLASMODIUM BERGHEI*

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Abstract: When *Anopheles stephensi* infected with *Plasmodium berghei* by bite were kept at 21°C in darkness, degenerative changes occurred in 33.8% of oocysts, as contrasted to 1.5% of such changes in mosquitoes kept at 21°C with 12-hr light period. Temperature shift of infected mosquitoes from 21°C for 24 hr to 25°C and 28°C increased the degeneration rate of oocysts to 87.8% and 85.8%, respectively, and inhibited oocysts maturation. *P. berghei* ookinetes were cultured from gametocytes of infected hamster blood and purified by discontinuous Percoll gradient, using the modified procedure described by Munderloh and Kurtti (1987). Suspensions of purified ookinetes (1×10^6/m*l*) fed through membrane feeders permitted stable infection of *A. stephensi*. Mosquitoes fed purified ookinetes in phosphate buffered saline (PBS) and then maintained only with sugar, were able to produce infective sporozoites. Fetal bovine serum, mammalian cell medium F-12, and hamster red blood cell, added to PBS, improved infectivity of ookinetes to mosquitoes and decreased degenerative changes during oocyst development. The system of feeding purified ookinetes in simple chemical solution, PBS, would help to gain more information about the transformation of ookinetes to oocysts.

INTRODUCTION

Most of the life cycle of the rodent malaria parasite, *Plasmodium berghei* can be studied in *in vitro* culture systems: exoerythrocytic cycle in liver (Hollindale *et al.*, 1983), erythrocytic cycle in blood (Janse *et al.*, 1984), gametocytogenesis (Mons *et al.*, 1985) and ookinete formation (Weiss and Vanderberg, 1977; Janse *et al.*, 1985a, b; Sinden *et al.*, 1985).

The transformation of ookinetes to oocysts and the development of oocysts have not yet been studied in an *in vitro* system. Progress in cultivation of ookinetes and their development to mature oocysts has been hampered by the lack of information on the biophysiological conditions of sporogony in anopheline mosquitoes. We report the effect of environmental temperature and darkness on sporogonic development of infected *Anopheles stephensi* and the nourishing effect of the feeding solution of purified *P. berghei* ookinetes as determined by examination of the number and degenerative changes of oocysts in mosquito midguts.

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Materials and Methods

Parasites and hosts:

The *Plasmodium berghei* (strain ANKA) —*Anopheles stephensi*—golden hamster (*Mesocriecetus auratus*) complex was maintained as described by Vanderberg *et al.* (1968), and used for experiments.

Usually hamsters, 17 to 24 days old, were inoculated with *P. berghei.* Older hamsters than these were injected with phenylhydrazine HCl (100 mg/kg) four days before infection (Janse *et al.,* 1985b).

For infection, 40-50 female mosquitoes 3-7 days old, were collected from a stock colony into small mosquito cages, using disposable 350 m*l* plastic caps. Infected mosquitoes were maintained at 21°C with a daily cycle of 12 hr of light and darkness on 5% sucrose provided on dental cotton roll.

Media:

The ookinete culture medium consisted of Ham's F-12 medium (GIBCO) without $NaHCO_3$, supplemented with 10% heat-inactivated fetal bovine serum (FBS: GIBCO), 25 mM BICINE. The pH of the medium was adjusted to 8.0 with 1N NaOH. The culture medium F-12, supplemented with 10% FBS, 25 mM HEPES and 0.2% $NaHCO_3$, and adjusted to pH 7.3 with 1N NaOH (washing medium), was used for washing ookinetes, for dissecting mosquito midguts and for diluting blood and 90% Percoll.

Culture and harvest of ookinetes:

Ookinetes were cultured and harvested according to the modified procedure described by Munderloh and Kurtti (1987). One m*l* of the parasitized blood containing approximately 15 units of heparine/m*l* was inoculated into petri dishes (100 mm diameter) (Falcon) containing 10 m*l* of ookinete culture medium and incubated at 21°C for 20-22 hr to develop ookinetes.

Purification of ookinetes:

Isotonic 90% Percoll was prepared by adding 1 volume of 10× concentrated medium 199 (GIBCO) to 9 volumes Percoll (Sigma). This was further diluted to 45% and 36% with washing medium. The above cultures were collected into centrifuge tubes in an ice bath and centrifuged at 150×*g* for 8 min at 4°C. Both supernatant and upper greyish layer on the sediment were used to collect ookinetes. The supernatants were centrifuged at 175×*g* for 10 min and the sediment were resuspended in 5 m*l* of 36% Percoll. The upper greyish layer of the sediment were collected into centrifuge tubes containing 10 m*l* of washing medium and also centrifuged at 175×*g* for 10 min. Then again, the upper greyish layers were carefully collected and resuspended in 36% Percoll. Both 36% Percoll suspensions were separately overlayed on 5 m*l* of 45% Percoll and then 3 m*l* of washing medium was overlayed on each suspension. The preparations were spun at 150×*g* for 5 min and then for another 12 min at 700×*g*. The interfaces between the 45% and 36% Percoll were removed with a Pasteur pipet, and the number of ookinetes and contaminated red blood cells (RBC) were counted in a hemocytometer. Samples containing RBC fewer than 30 per ookinete were washed once and resuspended with described membrane feeding solutions, then used for feeding.

Effect of environmental temperature and darkness on sporogonic development:

Mosquitoes in 2 small cages were allowed to feed on an infected hamster for 10 min at 21°C and then mosquitoes in other 2 cages were fed on the same hamster for 20 min at 21°C.

After acquisition of the infected blood, mosquitoes in one cage was kept at 21°C with 12-hr light period as a standard control. Some cages were kept at 21°C for 24 hr and then kept at 25°C or 28°C with 12-hr light period, respectively. Other cages were kept at 28°C with 12-hr light period or at 21°C in darkness throughout the experiment.

Infected mosquitoes were dissected on day 11 post infection (PI) (experiment 1) and day 12 PI (experiment 2 and 3). The dissected midguts were examined with an inverted phase-contrast microscope at 360× magnification for the presence of oocysts.

Sporozoite infections of hamsters were carried out on day 15 PI in experiment 1 and 2, and day 17 PI in experiment 3. In the experiment 1 and 2, anesthetized hamster pups were placed on the top of the mosquito cages. The infected mosquitoes were allowed to feed through the nylon net of the cage on the underside of the hamster for 20 min. In the experiment 3, each mosquito was cut in half at the junction of the thorax and abdomen. The head-thoraxes of mosquitoes containing mature oocysts in the abdomen were kept in an Eppendolf containing the washing medium, 0.2 to 0.5 m*l* in an ice bath according to the number of mosquitoes dissected, and crashed with a round head of a spatula, after all mosquitoes were examined for the presence of oocysts. The suspension was centrifuged at $150 \times g$ for 2 min to remove the large mosquito debris. When the number of sporozoites in the supernatant could be counted in a hemocytometer, 1×10^3 sporozoites were used for infection of a hamster by intracardial inoculation carried out under sodium pentobarbital anesthesia.

Table 1 Effects of environmental temperature and darkness on sporogonic development of *Plasmodium berghei*

Environmental condition[a)]	No. of experiment	% Infected (No. examined)	No. of oocysts/ infected mosquito±SD	Degeneration rate(%) of oocysts (average)	Infectivity of sporozoites (No. of mosquitoes used)
21°C	1	100.0 (15)	30.2±33.9	0	n.c.[b)]
	2	44.0 (18)	16.4±42.3	3.1	+(18)
	3	100.0 (8)	48.1±25.2	1.3 (1.5)	+ (3)
21°C in darkness	1	91.7 (12)	52.9±26.6	24.4	+(17)
	3	100.0 (9)	43.0±27.6	43.2 (33.8)	+ (7)
Temperature shift from 21°C to 25°C	1	100.0 (5)	19.6±11.3	87.8	n.c.
Temperature shift from 21°C to 28°C	1	100.0 (7)	22.0±22.7	81.8	− (8)
	2	66.6 (24)	19.0±32.5	86.2	−(32)
	3	90.6 (11)	19.8±15.9	89.4 (85.8)	− (2)
28°C	2	0 (23)			
	3	0 (15)			

a) Mosquitoes were kept with 12-hr light period except mosquitoes at 21°C in darkness.
b) Sporozoite infection was not checked.

If the number could not be counted, all the supernatant was used for infection of a hamster. To examine infectivity of sporozoites, the final examination of parasitemia was carried out on day 14 PI.

Nourishing effect of feeding solutions of purified ookinetes on the sporogonic development:

A. stephensi was infected by membrane feeding of purified ookinetes (1×10^6/m*l*) to study the essential nourishment of blood on sporogony of *P. berghei*. Phosphate buffered saline (PBS: 8.0 g of NaCl, 0.2 g of KCl, 1.15 g of Na_2HPO_4 and 0.2 g of KH_2PO_4 per litter) was used as non nourishment. Five per cent glucose and washing medium were used for feeding ookinetes as moderate nourishment. A experiment of mosquitoes infected with ookinetes in PBS and in 5% glucose were maintained on 5% glucose in stead of 5% sucrose. Heparinized fresh hamster blood was diluted two times with washing medium and used for feeding ookinetes as blood meal (complete nourishment), because a small volume of whole blood easily evaporates during feeding and because it is too sticky for mosquitoes to engorge. RBC of heparinized hamster blood (1 volume) were washed once with cold PBS (100 volumes) and resuspended with PBS (1 volume) as RBC suspension.

To study the nourishing effect of blood component on sporogonic development of purified ookinetes, PBS was supplemented with each 10% of hamster RBC suspension, FBS as serum component and hamster whole blood. Each feeding solution was supplemented with 10 mM of $NaHCO_3$, a phagostimulant for anopheline mosquitoes (Galun *et al.*, 1985; Yano *et al.*, in preparation).

Feeding of ookinetes to mosquitoes:

Purified ookinetes were resuspended in 0.5 to 1.5 m*l* of each feeding solution to obtain the concentration 1×10^6 ookinetes per m*l*.

Water-jacketed membrane feeders (2.6 cm in an inside diameter) (Rutledge *et al.*, 1964) fitted with a Baudruche membrane (Long and Long, Belleville, New Jersey) were used for

Table 2 Nourishing effect of feeding solution of purified *Plasmodium berghei* ookinetes on the sporogonic development

Feeding solution	Dissecting time (days)	% Infected (No. examined)	No. of oocysts/ infected mosquito±SD	Degeneration rate (%)	Infectivity of sporozoites
5% Glucose	12-18	12.1 (33)	1.3± 0.5	100.0	n.c.[a]
PBS	8-10	40.8 (71)	3.2± 3.7	4.3	n.c.
	12-18	44.1 (183)	4.3± 5.6	19.0	4/5[b]
PBS+10% FBS	8-10	68.4 (19)	18.9±20.8	0	n.c.
	12-18	87.0 (46)	29.5±29.5	18.6	2/2
Washing medium	8-10	45.5 (11)	5.4± 5.9	0	n.c.
	12-18	76.3 (76)	6.9± 6.8	11.0	6/6
Blood meal	8-10	92.3 (26)	42.5±41.1	4.8	n.c.
	12-18	87.4 (143)	31.6±34.7	7.9	5/5

a) Sporozoite infection was not checked.
b) No. of infection/No. of trials.

Table 3 Nourishing effect of blood component in the feeding solution of purified *Plasmodium berghei* ookinetes on the sporogonic development

Feeding solution	% Infected (No. examined)	No. of oocysts/ infected mosquito±SD[a)]	Degeneration rate (%)
PBS+10% FBS	100.0 (31)	36.3±29.8	17.6
PBS+10% RBC suspension	95.7 (23)	25.8±35.6	11.6
PBS+10% Whole blood	100.0 (29)	34.2±27.4	22.0
Blood meal	94.7 (18)	38.9±33.0	12.9

a) The presence of oocysts was examined on day 14 PI.

feeding. Feeders were pre-warmed at 37°C in a 21°C-incubator. Mosquitoes in small cage were fed ookinetes in solution for 15 min. From day 8-10 PI, and 12-18 PI, the dissected midguts were examined for the presence of oocysts. Sporozoite infection of hamsters was carried out by intracardial inoculation of day 14-18 PI as described before.

Degenerative rate of oocysts:

The degenerative changes of oocysts proceeded from a few granules observed in the protoplasm of an oocyst, to coarse granules that filled the whole protoplasm of an oocyst. Finally, the protoplasm with coarse granules shrunk away from the oocysts wall and concentrated in the oocyst as an amorphous mass. Oocysts which under phase-contrast microscopic examination contained distinct coarse granules were counted as degenerative oocysts. Degeneration rate of oocysts was caliculated from total degenerative oocysts per all oocysts of an experimental group.

Results

Effects of environmental temperature and darkness on the development of malaria oocysts:

Table 1 summarizes the effects of environmental temperature and darkness on the development of malaria oocysts. The rate of infection and numbers of oocysts per mosquito did not differ significantly among mosquitoes kept at 21°C with 12-hr light period (21°C mosquitoes), at 21°C in darkness, and of temperature shift from 21°C for 24 hr to 25°C (21°C to 25°C mosquitoes) or to 28°C (21°C to 28°C mosquitoes). Almost all oocysts of 21°C mosquitoes matured without degenerative changes but the oocysts of 21°C mosquitoes in darkness, 21°C to 25°C mosquitoes and 21°C to 28°C mosquitoes degenerated significantly during their maturation; the average degeneration rates of oocysts were 33.8%, 87.8% and 85.8%, respectively, in contrast to the 1.5% of 21°C mosquitoes. Infective sporozoites were recognized both in mosquitoes kept at 21°C with 12-hr light period and those kept in darkness. Mosquitoes that were shifted from 21°C for 24 hr to 25°C or 28°C could not develop mature oocysts with sporozoites. In none of the surviving mosquitoes kept at 28°C with 12-hr light period could any development of oocysts be recognized in their midguts.

Nourishing effect of feeding solutions of purified ookinetes on the sporogonic development:

Mosquitoes fed purified ookinetes in PBS developed infective sporozoites but the infective rate and the number of oocysts per mosquito were lower than those fed ookinetes in PBS supplemented with 10% FBS, ookinetes in washing medium or in blood meal (Table 2). Addition of 10% FBS to PBS improved the infection rate, 40.8% in day 8-10 PI and 44.1% in day 12-18 PI to 68.4% and 87.0%, respectively, and the number of oocysts per mosquito, 3.2±3.7 oocysts in day 8-10 PI and 4.3±5.6 oocysts in day 12-18 PI to 18.9±20.8 and 29.5±29.5, respectively.

Changes of PBS+10% FBS to washing medium (F-12+10% FBS+25 mM HEPES+0.2% $NaHCO_3$) decreased the degenerative rate, from 18.6% to 11.0% but the infected rate was not significantly different. Mosquitoes fed ookinetes in blood meal had the highest infection rates, 92.3% on day 8-10 PI and 87.4% on day 12-18 PI.

Young oocysts on day 8-10 PI of mosquitoes fed ookinetes in PBS with 10% FBS and washing medium did not degenerate but some later oocysts of mosquitoes fed ookinetes in all five kinds of solutions contained degenerative granules; the degenerative rates were 19.0% in PBS, 18.6% in PBS+10% FBS, 11.0% in washing medium and 7.9% in blood meal.

The infection rate of mosquitoes fed ookinetes in 5% glucose was very low, 12.1% of 33 mosquitoes in two experiments. The number of oocysts per mosquito was 1.3±0.5 and all oocysts were degenerated.

The addition of 10% RBC suspension to PBS decreased the degenerative rate of oocysts as did the blood meal (Table 3).

The number of oocysts in mosquitoes fed ookinetes in PBS+10% RBC suspension was the lowest, 25.8±35.6 in contrast to 36.3±29.8 in PBS+10% FBS, 34.2±27.4 in PBS+10% whole blood and 38.9±33.0 in blood meal, respectively.

Discussion

The temperature range which permits *P. berghei* ookinete formation in the natural vector *A. dureni,* is 18°C to 21°C (Yoeli, 1965). In the experimental vector, *A. stephensi,* oocysts did not develop at 28°C (Table 1). When ookinetes were allowed to form at 21°C for 24 hr after feeding parasitized blood, and then the environmental temperature was shifted to 25°C or 28°C, *A. stephensi* could develop young oocysts at 25°C and 28°C, but no mature oocysts formed at these temperatures.

Mosquitoes exposed to high temperature fail to develop sporozoites of *P. berghei* (Vanderberg and Yoeli, 1966). Thus the oocysts of 21°C to 25°C mosquitoes and 21°C to 28°C mosquitoes were highly degenerated during their later developmental stage. Oocysts without apparent degenerative changes were smaller and their development seemed to be retarded. Some had cloudy protoplasms with fine granules, indicating the start of degenerative changes. The damaging action of high temperature would begin before sporozoite budding, for there were no oocysts containing sporozoites.

In darkness, mosquitoes remained motionless in the cages. The degenerative changes of oocysts of 21°C mosquitoes in darkness were high. We assume that poor nourishment of mosquitoes, which did not feed on the 5% sugar solution in darkness was responsible for this finding. The transformation rate from ookinetes to oocysts increased in mosquitoes in darkness, compared to mosquitoes with 12-hr light period. We assume that the motionless of mosquitoes was responsible for this finding.

Mosquitoes fed ookinetes in PBS could develop mature oocysts and infective sporozoites when they were provided solely 5% sugar or 5% glucose on cotton roll like avian malaria ookinetes (Rosenberg and Koontz, 1984) (Table 2). Blood components were not essential on the development from ookinete to sporozoite.

Mosquitoes fed ookinetes in 5% glucose were seldom infected and they developed only a small number of degenerative oocysts per mosquito. The reason for this seems to be the weakly acidic glucose solution and its unstable pH.

The serum component, FBS, supplemented with PBS improved the viability of ookinetes in membrane feeders, and increased infectivity and the number of oocysts per infected mosquitoes almost the same as blood meal. The ookinetes kept in PBS in an ice bath for 30 min lost significantly their infectivity to mosquitoes compared to ookinetes kept in washing medium that proved completely infective for mosquitoes (100% infective rate). The RBC with PBS improved the viability of ookinetes to a lesser degree than FBS and whole blood (Table 3) but supplemented the nourishment and decreased the degeneration rate of developing oocysts.

The oocyst maturation in mosquitoes fed ookinetes in blood meal preceded by more than one day the maturation in mosquitoes fed ookinetes in PBS, PBS+10% FBS and washing medium. The maturation of oocysts could explain the higher degeneration rate of oocysts in day 8-10 PI of mosquitoes fed ookinetes in blood meal, because the degenerative changes became dominant in late stages of oocysts.

There was a large difference between the degeneration rate of oocysts in mosquitoes fed directly by bite on an infected hamster (1.5%) and those fed *in vitro*-formed ookinetes in blood meal (7.9% in Table 2 and 12.9% in Table 3). The gametocytes acquired by mosquitoes by bite should transform to ookinetes within 20 hr not to be trapped within the peritrophic membrane (Orihel, 1975). Thus ookinetes penetrating the peritrophic membrane to the midgut basement membrane would be biologically stronger than those transformed later and trapped within the peritrophic membrane, and could grow to mature oocysts and sporozoites without degeneration.

The ookinetes acquired by mosquitoes through membrane feeders could easily reach the midgut basement membrane before the peritrophic membrane hardened and had no selection of biological strongness. Some ookinetes could not retain the capacity for maturation due to unknown factors.

The experimental transformation of ookinetes to oocysts *in vitro* remained unsolved. The system of feeding purified ookinetes in simple chemical solution, PBS, would help to gain more information about it.

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Plasmodium berghei の sporogony について，蚊の飼育温度と純化 ookinete の発育に対する membrane feeding 液の影響

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Plasmodium berghei（ANKA 株）―シリアンハムスター―*Anopheles stephensi* の系を使用した。*P. berghei* 感染ハムスターを吸血した *A. stephensi* での ookinete 形成には，蚊の飼育温度を21°Cに保つことが必須条件であった。吸血蚊を21°Cの暗闇に保つと oocyst 形成は増えたが，oocyst の発育中に33.8%が変性を起こした。吸血後，*A. stephensi* を通常の飼育温度28°Cで飼育すると oocyst の形成は全く見られなかったが，吸血後24時間21°Cに保った後28°Cで飼育したものでは，oocyst の発育は見られたが，sporozoite への発育は阻害された。

P. berghei 感染ハムスター血を，10% FBS，25 mM Bicine 加 Ham's F-12 培地（pH 8.0）で約10%に希釈し，21°Cで22―24時間培養し，ookinete を得た。Percoll gradient（0～36%～45%）で純化した ookinete（1×10^6/m*l*）を，5%グルコース，PBS，PBS-10% FBS，F-12-10% FBS，ハムスター全血に再浮遊し，membrane feeding 法で蚊に感染させた。血液成分を使用しなくても，PBS を feeding 液として ookinete を蚊に感染させた後，5%蔗糖あるいは5%グルコースで飼育して，感染力のある sporozoite を得た。その場合の低い感染率(44.1%)，oocyst 数（4.3個）は，PBS に 10% FBS を添加することにより感染率（87.0%），oocyst 数（29.5個）とを，全血を feeding 液に使用した場合とほぼ同じ程度に回復させ得た。赤血球を全血の10%，PBS に添加した場合は，FBS 添加より感染率，oocyst 数は劣ったが，oocyst の変性率は低下した。FBS 添加による感染率，oocyst 数の回復効果は，oocyst 発育過程での栄養供給よりも，membrane feeding 中の ookinete の viability の低下を防ぐ為と思われる。赤血球には，oocyst 発育過程での栄養供給効果があると思われ，oocyst の変性率を低下させた。

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Research note

SURVEY OF *PLASMODIUM FALCIPARUM* IN MAKURDI, NIGERIA

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Studies on different aspects of *Plasmodium falciparum* have been reported by several workers (Dietz *et al.*, 1974; Aderounmu *et al.*, 1981; Ibeziake *et al.*, 1980; Krafsur *et al.*, 1978; Okeahialam *et al.*, 1972). Current conservative estimate puts the annual mortality from malaria among Nigerians as 100,000 according to the press note of Enwonwu (1983). Though much work on *P. falciparum* has been conducted in different parts of Nigeria, no attempt has so far been made to report the prevalence of malaria from the studied area. Therefore the present report is focussed on the results of a parasitological blood examination of individuals from local schools and local clinics in North Bank area of Makurdi, Benue, Nigeria during a period of three months (June-August 1984).

North Bank area is on the banks of River Benue and has a tributary River Guma on one side. It has a tropical savannah climate and there are two marked seasons, the wet season (April to October 40-60" rainfall) and dry season (October to April 0-10" rainfall). The age (0-30 years) and sex of the individuals (1,001) including the pregnant women were recorded at the time of blood sample collection. The blood smears were stained with Giemsa's for detecting the parasites. The parasite in positive slides and age groups were expressed according to the code followed by WHO (Black, 1968).

Results are summarised in Table 1, *P. falciparum* was the only prevalent species in the area and the infection rate was 1.2% in the studied population. Infections with *P. malariae, P. ovale* and *P. vivax* were not detected. So there were no mixed infections. Parasite rate of *P. falciparum* was 6.9% in the whole population being a measure of malaria prevalent in the area which was at its maximum.

Children between 0-5 months old were least affected, which may be due to parental protection. It has been reported that congenital infections are rare (McGregor, 1978). A definite indication of recent transmission of malaria in the area was known by the significant infant parasite rate which was 5.6%. It has been recently shown that *P. falciparum* invades cells which contain Haemoglobin F (HbF) to a greater degree than cells which contain Haemoglobin A (HbA) (Wilson *et al.*, 1977). The infection rate in the second year of life was less than that in the first year of life in the present survey, similar to the findings of Dar es Salaam (Okeahialam *et al.*, 1972), since the suppressive action of drugs gives a suppressive cure of *P. falciparum* (Bruce-Chwatt, 1982). Maximum effect was recorded in the age groups 2-4 years (7.7%). The present findings revealed that the parasite rate in children (1-14 yrs) was high (22.45%) while in adults (15-30 yrs) was low (13.1%), since immunity to malaria

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increases with age. In a survey conducted among the inhabitants of Ethiopia it was estimated that the parasite rates were two folds greater among children than adults (Krafsur *et al.*, 1978). Okeahialam *et al.* (1972) reported at Dar es Salaam, that more than two third of the children admitted were less than 2 years of age, and there were more boys than girls. But in the present findings, more than two third of children examined belonged to the age group 2-14 years and there were more girls than boys. It has been noted that females are much more frequently infected, while more males were infected than females as reported by Dutta *et al.* (1975).

The high parasite rate in women (15-30 years) when compared to the other separate age groups, indicates that placental malaria is a frequent event in pregnancy (McGregor, 1978). There appeared to be a decrease in parasite densities with age and the parasite density for the studied population was 3.6.

This preliminary parasite survey, however reports the maximum prevalence of malaria in the studied area being the wet season, and the prevalent parasite species in *P. falciparum* in Nigeria while infections with *P. malariae* are low and with *P. ovale* and *P. vivax* are scarce.

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Table 1 *Plasmodium falciparum* survey-Consolidated report

Age Groups	No. of persons examined		Blood examinations positive slides		Parasite rate		Infection rate for *P. falciparum*	Mean parasite density
	Males	Females	Males	Females				
0-11 months (infants)	23	31	2	1	5.6%		0.3%	6.48
12-23 months (children)	38	20	1	1	3.4%		0.2%	4.98
2-4 years (pre school children)	41	36	3	3	7.7%	22.45%	0.6%	3.24
5-9 years (juveniles)	177	220	11	17	7.05%		2.8%	2.76
10-14 years (adolescents)	113	119	2	8	4.3%		0.9%	1.63
15-30 years (adults)	12	171	—	24	13.1%		2.3%	2.53
Total	404	597	19	54				
Mean					6.9%		1.2%	3.60
SD					(3.5)		(1.1)	(1.8)

Locality: North bank area of Makurdi; Benue State; Nigeria.
Date: June-August, 1984. Season: wet

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Case report

LOCALIZATION OF HEPATITIS B SURFACE ANTIGEN IN THE PANCREAS AND LYMPH NODES

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Abstract: An autopsy case of syphilitic aortic aneurysm with an infection of hepatitis B virus in a 65-year-old woman is reported. Specifically, hepatitis B surface antigen (HBsAg) was revealed in the pancreatic acinar cells and histiocytes of retroperitoneal pancreatic lymph nodes as well as in part or nearly all of the cytoplasm of scattered hepatocytes by orcein and immunoperoxidase methods. These findings suggest that hepatitis B viurs may replicate in the pancreas. It was not clear whether HBsAg in the lymph nodes is related to infectiveness or not. The present study further confirms the previous reports of extrahepatic localization of HBsAg.

INTRODUCTION

The liver is thought to be the primary site of hepatitis B viral replication and synthesis. Although hepatitis B virus antigens have been extensively revealed in the hepatocytes, hepatitis B surface antigen (HBsAg) and/or its related immune complexes have been showed as extrahepatic localizations in the renal glomeruli (Brzosko *et al.*, 1974; Nowoslawski *et al.*, 1972), vascular wall (Michalak, 1978), and pancreas (Shimoda *et al.*, 1981; Yoshimura, *et al.*, 1981). Localization of HBsAg in hepatocellular carcinoma has been reported (Shikata, 1973; Nayak and Sachdeva, 1975; Nazarewicz *et al.*, 1977; Wu, 1979; Senba, 1981, 1982; Kawano, 1983). Thus, present evidence indicates that humoral immune mechanisms in relation to HBsAg are involved even in extrahepatic lesions. Histological location of HBsAg in the lymph nodes has not well been recognized.

We have observed morphologic localization of HBsAg in extrahepatic tissues by immunohistochemical method. The present postmortem case may give some evidence to suggest that hepatitis B virus replicate in extrahepatic tissues.

CASE REPORT

A 65-year-old woman was admitted to Nagasaki University Hospital for treatment of aortic aneurysm. The patient died of rupture of aortic aneurysm before operation and

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laboratory examinations of hepatitis B virus and liver functions. At autopsy, aortic aneurysm, 13 cm in diameter and located at the site 2 cm from the aortic valve, with endarteritis and periarteritis of the adventitial vessels, and vasa vasorum surrounded by lymphocytes and plasma cells of the aorta, suggesting syphilitic aortitis, was observed. The liver showed histologically chronic hepatitis with periportal fibrosis and lymphocytic cell infiltration.

Tissue specimens of all organs inculding liver, pancreas, and lymph nodes were taken at autopsy and fixed in 10% formalin. After ordinary methods of histological preparations, paraffin blocks of specimens were cut at 4 μ, and stained with histochemical methods using orcein (Merck, Art. 7091, Lot 8529084) (Senba, 1982) and immunoperoxidase methods (DAKO PAP KIT: K523, Lot. 063-3) for HBsAg.

HBsAg was revealed in numerous hepatocytes by orcein and immunoperoxidase methods. Furthermore, HBsAg was observed in the acinar cells of pancreas (Fig. 1), and histiocytes of retroperitoneal pancreatic lymph nodes (Fig. 2) by both orcein and immunoperoxidase methods. No particular lesions of the pancreas and lymph nodes were observed, microscopically. Any organ specimen examined other than liver, pancreas and retroperitoneal pancreatic lymph nodes did not show HBsAg.

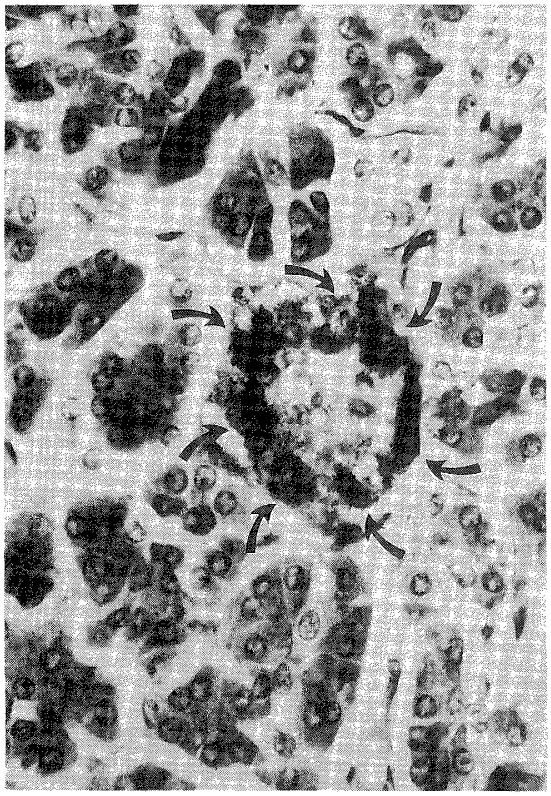

Figure 1 HBsAg is localized in the cytoplasm of some acinar cells of the pancreas (arrows). (Immunoperoxidase method, counterstained with hematoxylin, original magnification, ×400)

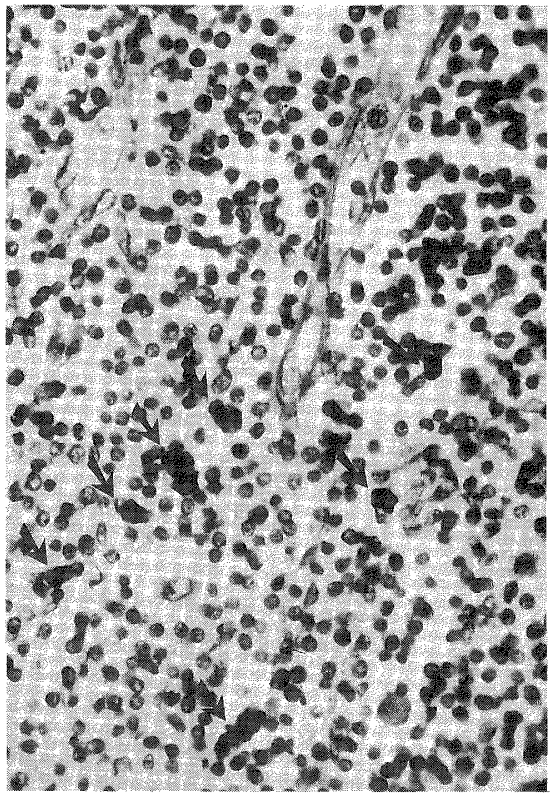

Figure 2 Cytoplasmic positive staining for HBsAg in the histiocytes of lymph nodes (arrows). (Immunoperoxidase method, counterstained with hematoxylin, original magnification, ×400)

Discussion

HBsAg has been revealed in both intrahepatic and extrahepatic tissues. Hepatitis B virus infection could be associated with the production of tissue damage outside the liver. In various extrahepatic diseases, immune complex deposition appears to be responsible for disease manifestations. It has been reported that various immunological processes may be involved and that host immune responses to the viral antigens may determine tissue damage according to morphological appearances and immunopathological abnormalities.

The present study showed that there is a possibility of infectiveness and replication of hepatitis B virus or at least an affinity of HBsAg to cells not only of the hepatocytes but also the pancreatic parenchymal cells and histiocytes of lymph nodes. However, we were not able to get any observation of pathogenesis of the pancreas and lymph nodes in this case. Whether or not HBsAg in the histiocytes of lymph nodes is related to infectiveness was not clear.

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膵臓およびリンパ節内に存在するB型肝炎ウイルス表層抗原

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梅毒性大動脈炎に起因したと考えられる，大動脈瘤破裂により死亡した65歳女性の剖検例において慢性肝炎が見出され，オルセイン組織化学染色および酵素抗体法により，肝実質細胞の胞体のほかに，膵臓の腺房細胞胞体やリンパ節の組織球内にも，B型肝炎ウイルス表層抗原(HBsAg)が観察された。HBsAgの存在する膵臓およびリンパ節の組織内において，病原体や異物などに起因する反応性所見は，見出せなかった。このことはB型肝炎ウイルスによる感染は，肝臓と肝臓以外の組織において，生体防御が異なるものと思われるが，肝臓以外におけるHBsAgの病態学的意義は，今後検討しなければならない。B型肝炎ウイルスは，肝実質細胞のほかに膵臓実質細胞にも存在しうることが，今までにも示唆されているが，本症例はこのことをいっそう裏付けるものである。なおHBsAgがリンパ節内に明らかに存在したという剖検例の報告は，我々が調べた範囲ではこれまでのところ見当らない。

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