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PARAGONIMUS OHIRAI: DRUG-INDUCED CHANGES IN THE GASTRODERMAL CELLS AFTER TREATMENT WITH PRAZIQUANTEL AND BITHIONOL

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Abstract: The effects of the anthelmintic drugs, praziquantel (PZQ) and bithionol (BT), on the gastrodermis of the lung fluke, *Paragonimus ohirai* adults were observed *in vitro* and *in vivo*. The gastrodermis of the worm treated with PZQ *in vitro* showed an increase in the number of autophagic vacuoles, most of which enclosed cytoplasmic elements, and multivesicular bodies. *In vivo*, myelin-like membranous bodies were observed. Autophagic vacuoles appeared as residual bodies near the luminal surface of the cells. Abnormal Golgi complexes with dense cisternae were seen on day 3-7 post-treatment. Rough endoplasmic reticulum was linear, reduced in size and numbers and partly disintegrated. The gastrodermis of the worm treated with BT *in vitro* showed considerable variety of damage. The tall, columnar cells in the secretory phase were swollen and vacuolated. Short, dense, pyramidal cells in the absorptive phase appeared to be normal. Active phagocytosis was observed at the luminal surface of the gastrodermis after 3 hr of incubation. Autophagic vacuoles and membranous whorls or vesicles were seen in the cells. Cellular breakdown was pronounced after 3 hr of incubation. *In vivo*, autophagic vacuoles were present near the apex of the cells. Cellular breakdown was conspicuous on day 7 post-treatment. These results suggest that autolytic breakdown of the gastrodermis induced by both drugs probably contributed to the eventual death of the parasite.

INTRODUCTION

There have been a number of reports dealing with the properties of anthelmintic drugs. Praziquantel (PZQ) is an anthelmintic with a broad spectrum of activity against parasitic flatworms including schistosomes, lung flukes of the genus *Paragonimus*, and cestodes. *In vitro* and *in vivo* studies on the effects of PZQ have shown that it causes contraction of musculature, a rapid and extensive vacuolation of the tegument, and destruction of female reproductive organs (Shaw and Erasmus, 1987, 1988; Becker *et al.*, 1980) as a result of altering intracellular Ca^{2+} homeostasis and metabolism including decreases in glucose uptake, lactate release and glycogen and ATP content (see Andrews, 1986). Bithionol (BT) has been used to treat paragonimiasis and also various diseases caused by cestodes (see Yokogawa, 1964). This drug inhibits glycolytic and oxidative metabolism, resulting in the destruction of the morphological integrity of the tegument, subtegumental cells, and parenchymal cells of trematodes (Hamajima, 1973; Hamajima *et al.*, 1979).

Little information is available on ultrastructural changes in the gastrodermis of trematodes following treatment with anthelmintics. Only Clarkson and Erasmus (1984) and Shaw and Erasmus (1983) described the *in vivo* effects of some anti-shistosome drugs on the ultrastructure of *S. mansoni* gastrodermis, which is made of a syncytium.

The present study was done to investigate changes of the cellular gastrodermis of the rat lung fluke, *Paragonimus ohirai* adults, affected by PZQ and BT *in vivo* and *in vitro*.

MATERIALS AND METHODS

Metacercariae of *Paragonimus ohirai* were removed from crabs, *Sesarma* (*Halometopus*) *dehaani*, from the Maruyama River, Hyogo Pref., central Japan. Adult worms, 60-day-old, were recovered from experimentally infected albino female rats (Sprague-Dawley) and washed with Ringer's saline.

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Praziquantel (PZQ) and Bithionol (BT) were gifts of Shanghai Sixth Pharmaceutical Factory, Shanghai and Tanabe Pharmaceutical Co., Tokyo, respectively. PZQ was dissolved first in 100 μl of Cremophor EL and then diluted with the defined medium NCTC 135. Soluble BT was prepared by neutralization with NaOH. For *in vitro* study, worms were incubated for 1, 3, 8 and 20 hr in the NCTC 135 containing 3×10^{-4} M PZQ (pH 7.3) or 10^{-4}M BT (pH 7.3) at 37 °C. Controls consisted of NCTC 135 and 1 % Cremophor EL (pH 7.3) without drug. Streptomycin (100 IU/ml) and Penicillin (100 ug/ml) were added to the test and control media. For *in vivo* study, PZQ was administered to adult rats as a suspension in Cremophor EL at the dose level of 500 mg/kg body weight/day twice every other day prior to necropsy of the hosts. Control rats were given 1 % Cremophor EL. The rats were killed 1, 3 and 7 days after the final dose of the drug. BT was administered orally to 2 adult rats as an aqueous solution, at the dose level of 200 mg/kg body weight/day 3 times every other day before the necropsy. Controls consisted of distilled water given orally to infected rats. The rats were killed at the same day as in the PZQ test.

For transmission electron microscopy (TEM), the specimens were rinsed in NCTC 135 and fixed for 2 hr in 5 % chilled glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). The material was then postfixed for 2 hr with 1 % osmium tetroxide buffered to pH 7.3. After dehydration with an ethanol series the specimens were embedded in Quetol 812 (Nisshin EM., Tokyo). Thick sections were stained with toluidine blue O for light microscopy (LM). Ultrathin sections were double stained with uranyl acetate and lead acetate and viewed in a Hitachi H-500 electron microscope at 75 kV.

For cytochemical studies on acid phosphatase (AcPase) and thiamine pyrophosphatase (TPPase), the specimens were incubated for 3 and 8 hr in each test medium. After incubation, the worms were washed in 0.1 M cacodylate buffer (pH 7.4), fixed, sectioned and incubated in the reaction medium following the method of Fujino and Ishii (1988a). AcPase activity was detected by the modified Gomori (1952) method and TPPase activity was detected using the method of Novikov and Goldfischer (1961). Controls consisted of incubating mixtures without substrates, or adding 10 mM sodium fluoride to the incubation medium.

RESULTS

Control worms

The gastrodermis consisted of tall, columnar cells with numerous secretory granules of different density in the secretory phase (Fig. 1) and short, pyramidal cells including few secretory granules in the absorptive phase. Lipid droplets were seen apically. The cytoplasm of the gastrodermal cells in the secretory phase was filled with well-developed rough endoplasmic reticulum and secretory granules. Minute reaction deposits for AcPase were on secretory granules, endoplasmic reticulum and the surfaces of the lamellar projections. Reaction sites for TPPase were Golgi complexes and multivesicular bodies (not shown). These enzyme activities were different in the cells, probably reflecting different physiological conditions of the absorption-secretion cycle in the gastrodermal cells. There were no ultrastructural differences in the gastrodermis *in vitro* versus *in vivo*.

The effects of PZQ

The gastrodermis in the *in vitro* experiment appeared thinner than that of the control. The number of lipid droplets markedly increased and secretory granules decreased (Fig. 2). The number of autophagic vacuoles, which were partly reactive to AcPase (Fig. 3), increased in the cells by 3 hr of incubation. Many of these vacuoles contained cellular fragments such as mitochondria, lipid droplets and whorls of endoplasmic reticulum. Myelin-like membranous bodies were observed occasionally. Multivesicular bodies reactive to TPPase appeared by 8 hr incubation (Fig. 3 inset). Golgi complexes, which mostly formed vesicular, round, unusual structures, were seen. After 20 hr of incubation, endoplasmic reticulum and ribosomes were partly disintegrated and reduced in number in the cytoplasm of the cells (not shown). Some filamentous structures as seen in the gastrodermis of starved worms (see Fujino and Ishii, 1988a) occureed in the rough endoplasmic reticulum, and were most notable after 20 hr of incubation. The lamellar cytoplasmic projections and mitochondria appeared to be intact.

The gastrodermis *in vivo* appeared to be normal on day 1 post-treatment. The cytoplasm of the gastrodermal cells *in vivo* became granular and there appeared an area devoid of organelles on day 3 post-treatment. Autophagic vacuoles including cytoplasmic elements were seen (Fig. 4). Some of these vacuoles were near the apical region of the cells and opened to the gut lumen and residues from intracellular digestion appeared to be emptied into the gut lumen (Fig. 5). Myelin-like membranous whorles appeared (Fig. 6). Elongate, compact and electron-dense mitochondria were dispersed, and only small numbers of sectetory granules remained. Endoplasmic reticulum was linear, partly disintegrated and reduced in number (Fig. 7). Golgi

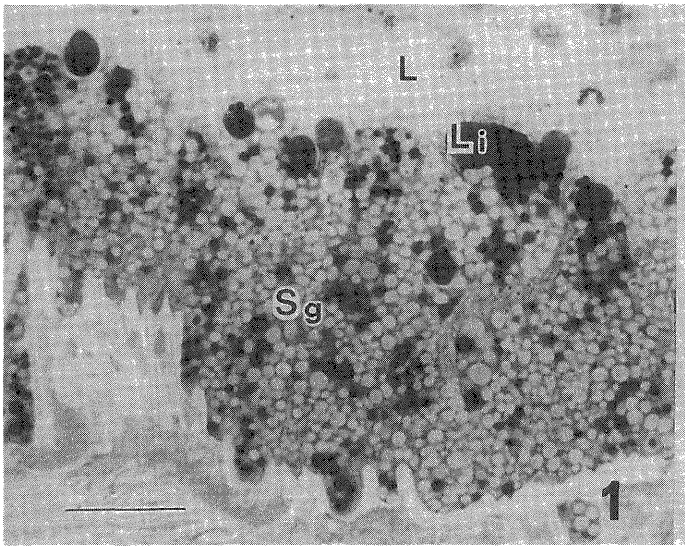


Figure 1 Cross section of part of the control gastrodermis in the secretory phase showing columnar cells with numerous secretory granules (Sg) and lipid droplets (Li). Stained with toluidine blue O. L: lumen. Bar=30 μm.

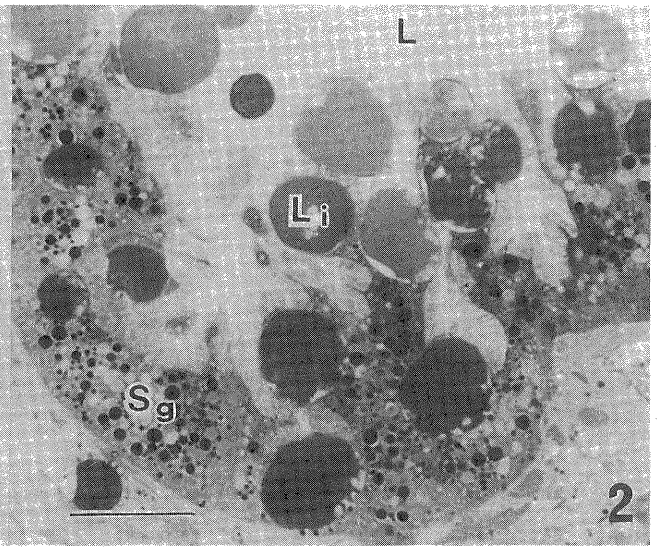


Figure 2 Cross section of part of the gastrodermis treated with PZQ for 8 hr *in vitro* showing fewer secretory granules (Sg) and increased numbers of lipid droplets (Li) than the control. L: lumen. Bar=30 μm.

complexes, whose cisternae were occasionally disorganized, separated, and filled with dense granular substance, were seen on days 3-7 post-treatment (Fig. 8b -c). The membranes of cisternae were ill-defined and no typical condensing vacuoles seen in the control (Fig. 8a) were observed. The basal plasma membrane of the gastrodermis was occasionally deeply infolded to entrap cellular elements like mitochondria and rough endoplasmic reticulum.

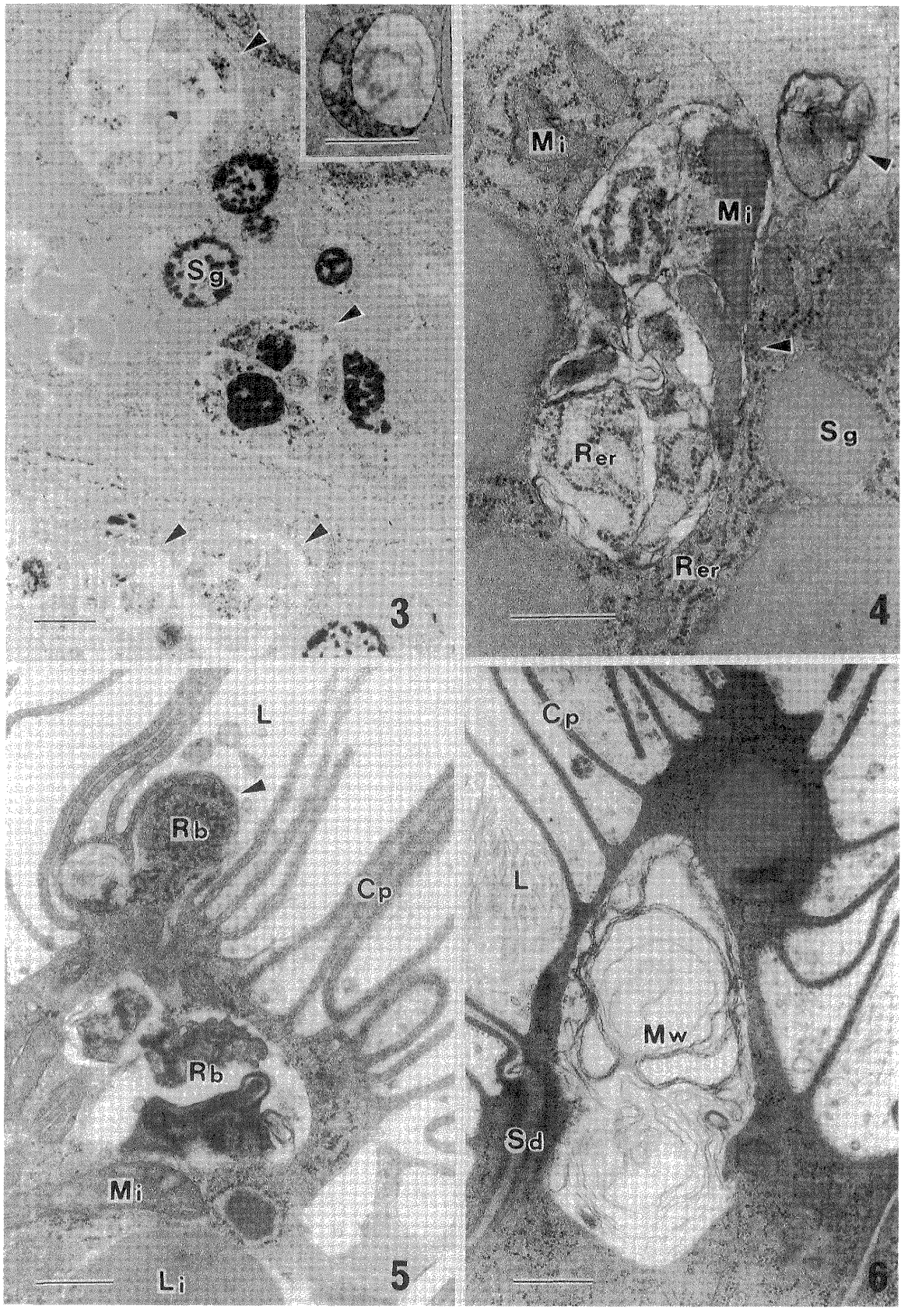
The effects of BT

The gastrodermal cells *in vitro* showed some damage after 3 hr of incubation. Tall, columnar cells in the secretory phase were swollen and vacuolated, and occasionally included lipid droplets and/or fluffy material inside (Fig. 9). Small, dense secretory granules whose peripheries were reactive to AcPase were seen basally in these cells. Short, dense, pyramidal cells in the absorptive phase appeared normal by LM (Fig. 9). Autophagic vacuoles and single or grouped membranous bodies, which were partly reactive to AcPase and TPPase, were seen throughout the cell after 3 hr incubation (Fig. 10). In some cells, numerous small vacuoles appeared. Active phagocytosis was observed at the luminal surfaces of the cells; the apical plasma membrane entrapped luminal food substances, invaginated deeply, and pinched off to make vacuoles in the cytoplasm of the cells (Fig. 11). Mitochondria were round, condensed, and electron-dense. In other cells, cellular breakdown was pronounced and the cytoplasm contained dense mitochondria, a few secretory granules and condensed or disintegrated rough endoplasmic reticulum. Numerous membranous whorls were distinct in the cytoplasm. Nuclei, situated close to the bases of the cells, were granular with condensed nucleoli (Fig. 12). The lamellar cytoplasmic projections appeared to be normal.

Autophagic vacuoles were observed near the apex of the cells on day 1 post-treatment *in vivo*. Filamentous structures were formed in the rough endoplasmic reticulum. Endoplasmic reticulum was disintegrated and reduced in number on day 3 post-treatment. Cellular breakdown was conspicuous on days 3 and 7 post-treatment although the plasma membranes, mitochondria, and some secretory granules and endoplasmic reticulum remained in the cells.

Discussion

No marked morphological differences were observed in the gastrodermal cells of *Paragonimus ohirai* treated *in vitro* versus *in vivo* with PZQ and BT. The most common ultrastructural feature of the cellular response to the treatment with PZQ and BT was an increase of autophagic vacuoles. Most of these vacuoles contained cell components like mitochondria and the endoplasmic reticulum. High levels of autophagy were also observed in the *in vivo* study of the anthelmintic drugs, Astiban, Hycanthone and Lucanthone, in the



Figures 3～6

gastrodermis of *Schistosoma mansoni* (see Clarkson and Erasmus, 1984). Similar phenomenon occurred under starvation *in vitro* in the gastrodermis of some trematodes (Bogitsh, 1973, 1975; Bogitsh and Ryckman, 1982; Fujino and Ishii, 1988a). Bogitsh (1975) reported a remarkable increase of Golgi complexes in the schistosome gastrodermis following treatment with Hycanthone *in vitro*. He mentioned that this drug produced certain of the effects characteristic of starvation by inhibiting the digestion of hemoglobin and that the effects of starvation were dramatically accelerated by the drug. Fujino and Ishii (1988a) also noted in the starvation test the increase of Golgi complexes in the gastrodermis of *P. ohirai*. However, marked increase of Golgi complexes, which are reactive to AcPase and TPPase, was not observed in the present study. It is possible that the drugs tested damaged the rough endoplasmic reticulum and blocked the formation or function of Golgi complexes, from which are derived lysosomal vesicles including hydrolytic enzymes. This idea seems to be supported by the present observations that the rough endoplasmic reticulum was partly disintegrated or reduced in number and only a few Golgi complexes, most of which were unusual in shape, and few lysosomes and vacuoles having hydrolase activity appeared in the gastrodermis of *P. ohirai*. Coles (1973) reported that schistosomes may become resistant to thioxanthenone drugs, and autophagic response may be important as a means of recycling damaged cellular components and conserving energy and resources. A similar mechanism in the trematode gastrodermis would allow toxic substances to be expelled directly into the gut lumen. These facts suggested that increased autophagy in the trematode gastrodermis is a common response to drug treatments regardless of different chemotherapeutic properties of the drugs as noted in schistosomes by Clarkson and Erasmus (1984). Bogitsh (1975) hypothesized that the infoldings of the basal plasma membrane of the gastrodermis are involved in encapsulation of organelles by engulfing portions of the cytoplasm.

Another common response after the drug treatments in the present study, is the appearance of myelin-like membranous whorls or vesicles. Shaw and Erasmus (1983) also noted large, membranous whorls and small, lucent vesicles in the gastrodermis of *S. mansoni* treated with PZQ *in vivo*. This phenomenon also occurred following worm starvation in the gastrodermis of *P. ohirai* (see Fujino and Ishii, 1988a) and some other trematodes (Bogitsh, 1973, 1975; Bogitsh and Ryckman, 1982).

Although most cellular changes induced by treatment with PZQ and BT were similar, the gastrodermal cells in the secretory phase were swollen and vacuolated when treated *in vitro* with BT, but not with PZQ. By 1 day post-treatment *in vivo*, cellular changes were not observed in the gastrodermis treated with PZQ, but some changes had occurred with BT treatment. Active phagocytosis was seen at the luminal surfaces of gastrodermal cells following 3 hr incubation in BT. Tests at different drug concentrations, therefore, would indicate whether morphological differences in the gastrodermis were due to differences in the effectiveness of PZQ versus BT.

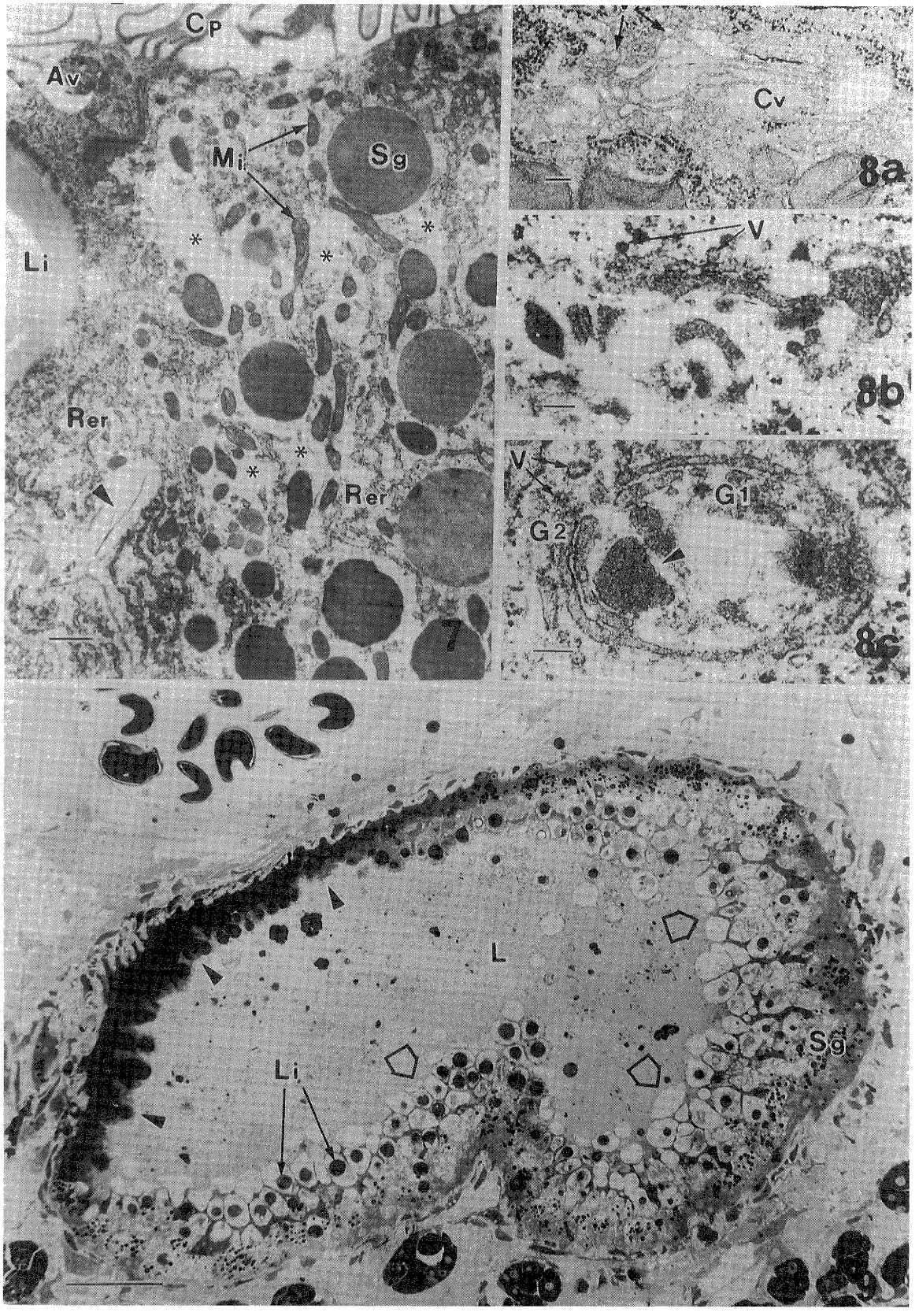
It was reported that PZQ decreased glucose uptake and lactate excretion (Andrews, 1986; Harder *et al.*, 1987). This would cause the alteration of glycolytic metabolism of the gastrodermis, resulting in the formation of autophagic vacuoles and membranous vesicles as seen in worm starvation. It has also been suggested that PZQ may alter Ca^{2+} homeostasis by interacting with membrane phospholipids (Harder *et al.*, 1988; Schepers *et al.*, 1988). As host body fluid *in vivo* or culture medium *in vitro* is imbibed through the pharynx into the gut lumen the luminal surfaces of the gastrodermis will come in contact with luminal substances including drugs. The change in ion permeability of the plasma

Figure 3 Gastrodermis of the worm treated with PZQ for 8 hr *in vitro*. AcPase reaction. Reaction deposits are on secretory granules (Sg), autophagic vacuoles (arrowheads). Bar=1 μm. Inset: Multivesicular body, TPPase reaction. Bar=0.5 μm.

Figure 4 Gastrodermis of the worm treated with PZQ *in vivo* on day 3 post-treatment. Autophagic vacuoles (arrowheads). Note cellular elements such as mitochondria and rough endoplasmic reticulum in the vacuole. Mi: mitochondrion; Sg: secretory granule, Rer: rough endoplasmic reticulum. Bar=0.5 μm.

Figure 5 Apical part of the gastrodermis of the worm treated with PZQ *in vivo* on day 3 post-treatment. A large vacuole of residual body (Rb) is seen near the apex of the cell. Another residual body (arrowhead) appears to be emptying its contents into the lumen (L). Cp: cytoplasmic projection; Li: lipid droplet; Mi: mitochondrion. Bar=0.5 μm.

Figure 6 Apical part of the gastrodermis of the worm treated with PZQ *in vivo* on day 3 post-treatment. A myelin-like membranous whorl (Mw) is seen. Cp: cytoplasmic projection; L: lumen; Sd: septate desmosome. Bar=0.5 μm.



Figures 7～9

membranes of the cells by the drug would initiate the gastrodermal destruction as was indicated in the tegument by Schepers *et al.* (1988). With regard to tegumental disruption, increased intracellular Ca^{2+} induced cytoskeletal disruptions and membrane blebbing (Klaassen and Eaton, 1991).

Clear, cellular differences in response to the drug effects appeared in the gastrodermis of *P. ohirai,* and this would be different from those in schistosomes, in which the gastrodermis is syncytial and no marked regional difference in the gastrodermis was noted (see Spence and Silk, 1970). The gastrodermis of *P. ohirai* in the secretory phase was apparently damaged after treatment with BT *in vitro,* in contrast, those in the absorptive phase appeared to be intact. Physiological activities of the cells in the secretory phase observed in some trematodes are believed to be high, but much less in the absorptive phase (Robinson and Threadgold, 1975; Fujino and Ishii, 1988b). BT was reported to inhibit enzymes concerning glycolytic and oxidative metabolism of *Paragonimus* species (Murakoshi and Moriya, 1968; Hamajima, 1973; Hamajima *et al.,* 1979). Therefore, the energy metabolism in the gastrodermis, especially in the physiologically active secretory phase, would be altered or inhibited by BT, and damages by the drug appeared more conspicuous in the cells of secretory phase than in those of the absorptive phase.

Active phagocytosis occurred at the luminal surfaces of the gastrodermis of *P. ohirai* treated with BT for 3 hr *in vitro.* Fujino and Ishii (1988c) described the presence of phagocytic invagination and vacuoles in the gastrodermis even in normal *P. ohirai.* It may be that phagocytosis becomes active during a certain time of incubation in compensation for the absorption of nutrients through the surfaces of the cells, which was inhibited by the drug. The mechanism for inducing phagocytosis remains to be elucidated.

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Figure 7 Gastrodermis of the worm treated with PZQ *in vivo* on day 3 post-treatment. Lamellar cytoplasmic projections (Cp) appear to be intact. The cytoplasm of the cell contains autophagic vacuoles (Av), myelin-like structure (arrowhead), condensed, electron-dense mitochondria (Mi), partly disintegrated rough endoplasmic reticulum (Rer), secretory granules (Sg) and lipid droplet (Li). There appeared an area (*) devoid of organelles in the cytoplasm. L: lumen. Bar=1 μm.

Figure 8a-c Golgi complexes of the gastrodermis. a, Control worm. Cisternae of Golgi are distended to form condensing vacuoles (Cv). Many small vesicles (V) can be seen in vicinity of emitting face. Bar=0.1 μm. b-c, Worms treated with PZQ *in vivo* on day 8 post-treatment. b, Separated cisternae with ill-defined membranes are filled with dense granular substance. Small vesicles (V) are seen near the cisternae. Bar=0.1 μm. c, Two adjacent Golgi complexes (G1, G2). Cisternae are dense and granular. A structure resembling a condensing vacuole (arrowhead) is present. V: vesicle. Bar=0.1 μm.

Figure 9 Cross section of the gut of the worm treated with BT for 3 hr *in vitro.* Most of the tall cells which are in the secretory phase (large arrows) are swollen and vacuolated, remaining lipid droplets (Li) inside and a few secretory granules (Sg) basally. Short, triangular cells in the absorptive phase (arrowheads) appear to be intact. L: lumen. Bar=1 μm.

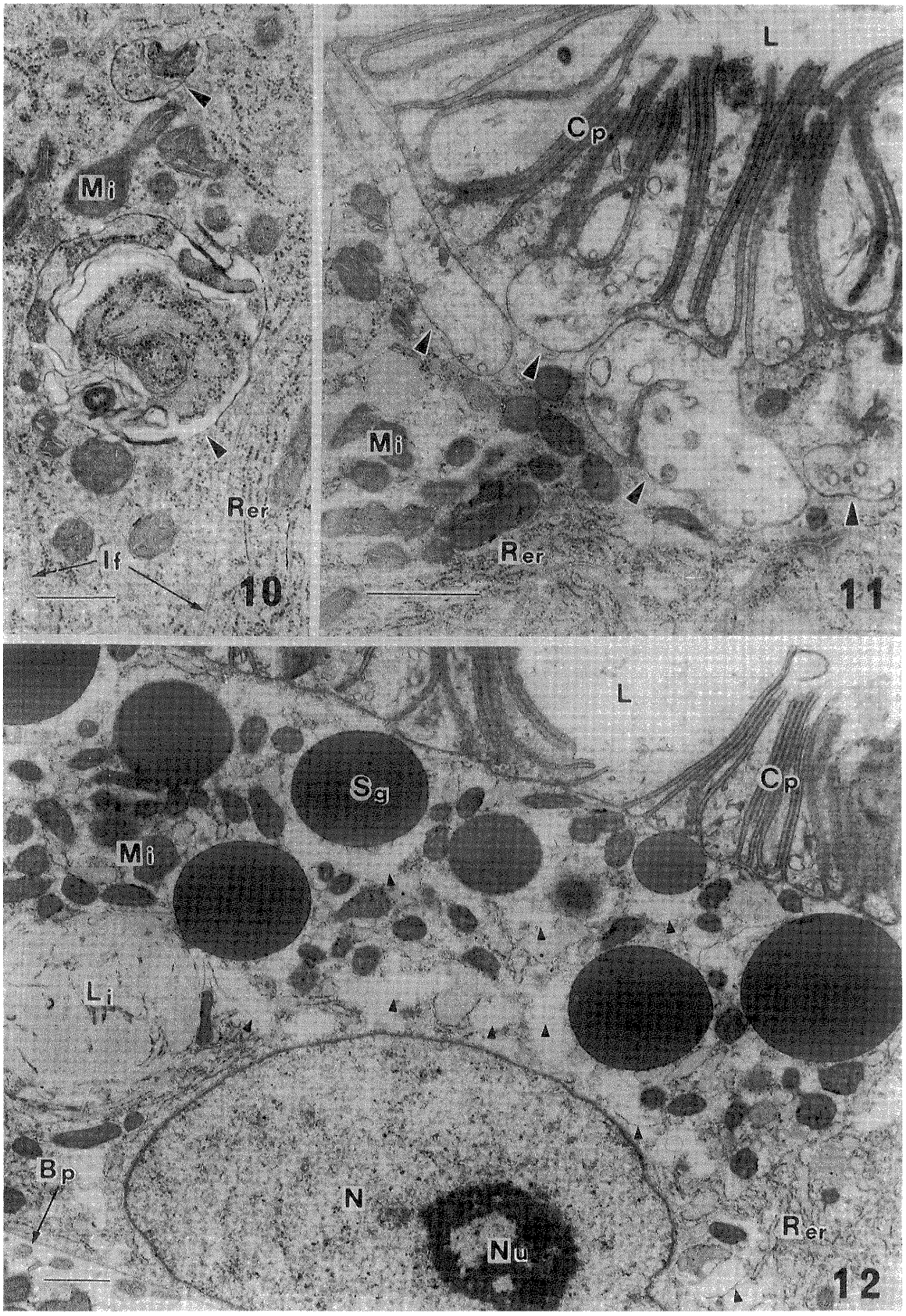

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Figure 10 Gastrodermis of the worm treated with BT for 3 hr *in vitro*. Autophagic vacuoles (arrowheads) appeared. If: infolding of basal plasma membrane; Mi: mitochondrion; Rer: rough endoplasmic reticulum. Bar=0.5 μm.

Figure 11 Apical part of the gastrodermis of the worm treated with BT *in vitro* for 3 hr, showing phagocytosis. The apical plasma membrane entraps luminal substances and is invaginated deeply into the cell (arrowheads). Cp: cytoplasmic projection; L: lumen; Mi: mitochondrion; Rer: rough endoplasmic reticulum. Bar=1 μm.

Figure 12 Gastrodermis of the worm treated with BT for 3 hr *in vitro*. The cell is flattened, the cytoplasm contains numerous membranous whorls (small arrowheads), dense, ovoid mitohcondria (Mi) and disintegrated rough endoplasmic reticulum (Rer) and secretory granules (Sg). An ovoid granular nucleus (N) with a condensed nucleolus (Nu) is situated near the base of the cell. Cp: cytoplasmic projection; Bp: basal plasma membrane; L: Lumen; Li: lipid droplet; Bar=1 μm.

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SEASONAL VARIATION OF BIRTHS IN A LOCALITY OF HOCHIMINH CITY, VIETNAM

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Received march 20 1994/Accepted may 15 1994

Abstract: Matsuda S (Department of Preventive Medicine and Community Health, School of Medicine, University of Occupational and Environmental Health, Japan, Yahatanishi-ku, Kitakyushu, 807, Japan). Seasonal variation of births in a locality of Hochiminh city, Vietnam

Seasonal variations of births in a community of Hochiminh city, Vietnam are analyzed by a Kolmogorov -Smirnov type statistics.

The excess in births is observed in the latter part of the year, but there is a difference in the timing of the peak of the first and subsequent births, that is, July to August for the first births and September and November for the subsequent births. This fact suggests that associated factors are different between the two parity groups. It does not seem that marriages and the lunar new-year holidays are important enough to create seasonal variations in conception in this population.

On the other hand, the traditional social belief of reproductive activity seems to play some role in creating the seasonality in births. And temperature hypothesis is also plausible for this population.

Seasonality of births in man have been documented extensively in the literature from the world (1-9, 11-23, 25-29) Roughly speaking, there are two types of theory to explain the seasonality of births; the socio-cultural theory which emphasizes cultural factors such as marriage, festivals, and agricultural cycle, and the biometeorological theory which stress on effects of various meteorological factors such as temperature, moisture, and light, on human reproductive activities. But there has been no definitive explanation for this phenomenon. A comparative study of different regions would be useful to yield an insight into the factors related to this phenomenon. Therefore, a number of studies in climatically and culturally different regions and a comparison of their results is desirable. To my knowledge, there has been up until now no published reports concerning the seasonality of births in Vietnam. From February to March 1993 I was fortunate to be able to make a field study to obtain some data on the seasonality of births in Vietnam. In this report, I present the results of this field study and the interpretations derived from it.

MATERIAL AND METHOD

Data source

The data are from the vital statistics registration system in Tanbinh district, which is located in the northern part of the Hochiminh city. Most of the inhabitants are involved in the secondary and tertiary industries. In Vietnam, all births have to be registered at the Population Committee of the basic administrative organization (village, town, etc). According to this registration, a series of health services are offered to mothers and children (i.e.: immunization) by the health staff of the local health station. During my study, I was able to obtain the birth data of 1992 in the 4th station of the Tanbinh district. The total number of births registered at this station during 1992 was 232 (Table 1).

Statistical analysis

The Kolmogorov-Smilnov type statistics were used for testing the departure from a uniform seasonal variation in birth number (10). This test is a test of goodness of fit. That is, it is concerned with the degree of agreement between the sample distribution and some special theoretical distribution. It determines whether

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Table 1 Monthly distribution of number of births in a community of Hochiminh city (1992)

	Jun	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
First births	7	8	9	7	6	6	13	14	8	4	4	2
Expected value	7.5	6.9	7.5	7.2	7.5	7.2	7.5	7.5	7.2	7.5	7.2	7.5
Subsequent births	10	16	13	8	8	9	11	7	18	17	14	13
Expected value	12.2	11.4	12.2	11.8	12.2	11.8	12.2	12.2	11.8	12.2	11.8	12.2
Total	17	24	22	15	14	15	24	21	26	21	18	15

$$\text{Expected value} = \frac{\text{Number of births in i th month}}{366} \times \text{Yearly total of births}^*$$

*: 88 for first births, 144 for the subsequent births

the sample values can reasonably be thought to have come from a population having the theoretical distribution. The statistical testing is concerning to whether the difference in two distribution, theoretical and observed, is likely on the basis of chance or not. The details of the procedure are presented in Table 2 and 3. In these tables, F(t) represents the cumulative distribution function on the assumption that there is a uniform seasonal distribution. Since the data are grouped into months, the cumulative distribution function F is a step function with 12 steps. In this study, the value of F at the end of January was 31/366: at the end of February, the value was (31+29)/366, because 1992 was a leap year. These values are represented by $F(1), F(2), \cdots, F(12)$. Similarly, the sample cumulative distribution function $F_N(i)$ $(i=1, 2, \cdots, 12)$ is also a step function with value n_i/N (n_i=number of births in i th month; $i=1, 2, \cdots, 12$; N=yearly total number of births). For example, $F_N(1)=7/88$ for the first births in this study. The V_N is defined as follows:

$V_N = \max(F_N(i) - F(i)) + |\min(F_N(i) - F(i))|$ $(1 \leq i \leq 12)$.

And $V_N\sqrt{N}$ is used as the test statistics. The significance of the test is evaluated according the table established by Freedman (10, see appendix).

RESULTS

Table 1 shows observed number of births and expected numbers both for first and subsequent births. In this table, an expected value for each month is calculated as follows:

expected value of i th month = (number of days in i month × yearly total number of births)/366,

Table 2 Results of the Kolmogorov-Smirnov type statistical testing for seasonal variations in a community of Hochiminh city (1992)
(First births)

Month	Frequency	Cummrative frequency	$F_{N(i)}$	$F_{(t)}$	$F_N - F$
Jan	7	7	0.0795	0.0847	−0.0052
Feb	8	15	0.1705	0.1639	0.0066
Mar	9	24	0.2727	0.2486	0.0241
Apr	7	31	0.3523	0.3306	0.0217
May	6	37	0.4205	0.4153	0.0052
Jun	6	43	0.4886	0.4972	−0.0086
Jul	13	56	0.6364	0.582	0.0544
Aug	14	70	0.7955	0.6667	0.1288
Sep	8	78	0.8864	0.7486	0.1378
Oct	4	82	0.9318	0.8333	0.0985
Nov	4	86	0.9773	0.9153	0.062
Dec	2	88	1	1	0
Total	88				

The expalnation of F and F_N is in the text.
$\text{Max}(F_N - F) = 0.1378$, $|\text{Min}(F_N - F)| = 0.0086$
$V_N = 0.1378 + 0.0086 = 0.1464$
$V_N\sqrt{N} = 0.1464 \times \sqrt{88} = 1.37$ $(0.05 < p < 0.10)$

Table 3 Results of the Kolmogorov-Smirnov type statistical testing for seasonal variations in a community of Hochiminh city (1992)
(Subsequent births)

Month	Frequency	Cummrative frequency	$F_{N(t)}$	$F_{(t)}$	$F_N - F$
Jan	10	10	0.0694	0.0847	−0.0153
Feb	16	26	0.1806	0.1639	0.0167
Mar	13	39	0.2708	0.2486	0.0222
Apr	8	47	0.3264	0.3306	−0.0042
May	8	55	0.3819	0.4153	−0.0334
Jun	9	64	0.4444	0.4972	−0.0528
Jul	11	75	0.5208	0.582	−0.0612
Aug	7	82	0.5694	0.6667	−0.0973
Sep	18	100	0.6944	0.7486	−0.0542
Oct	17	117	0.8125	0.8333	−0.0208
Nov	14	131	0.9097	0.9153	−0.0056
Dec	13	144	1	1	0
Total	144				

The explanation of F and F_N is in the text.
$\mathrm{Max}(F_N - F) = 0.0222$, $|\mathrm{Min}(F_N - F)| = 0.0973$
$V_N = 0.0222 + 0.0973 = 0.1195$
$V_N\sqrt{N} = 0.1195 \times \sqrt{144} = 1.43$ $(p < 0.05)$

Appendix: Percentiles of the distribution of $V_N\sqrt{N}$

Per cent	85%	90%	95%	99.00%
Percentile	1.21	1.29	1.41	1.66

Freedman, L.S. (1970)

where yearly total number of births=88 for the first births, and 144 for the subsequent births.

The first births show an excess in July to September and a following trough in the 4th part of the year. On the contrary, the subsequent births show the excess from September to November.

Table 2 and 3 show the monthly variation of birth numbers stratified by parity with the results of the Kolmogorov-Smirnov type statistical testing. The frequencies and cumulative frequencies are shown in the second and third columns of the tables. The fourth and fifth columns show the values of functions F_N and F. The final column shows the difference between the two values. The calculation of $V_N\sqrt{N}$ is shown at the bottom of the tables. In the first births, the excess births are observed in July and August. As $V_N\sqrt{N}$ value of 1.37 lies between 90% and 95%, the departure from a uniform seasonal variations is not significant at the 5% level. In the case of the subsequent births, the excess is observed in September and November and the value of $V_N\sqrt{N}$ is 1.43, which falls between 95% and 99%.

DISCUSSION

These results assume the existence of seasonal variations of births in the studied Vietnamese community. The excess in births is observed in the latter part of the year, but there is a difference in the timing of the peaks of the first and subsequent births; July to August in the first births and September to November in the subsequent births. This fact suggests that the associated factors of the two parity groups are different. This difference of peaks in seasonal variations between the first births and the subsequent births is also observed in the Japanese population (15-17). Unfortunately, other studies done in developing countries have not distinguished the first births and subsequent births. Such studies can easily cause a wrong interpretation of the results.

As I reported elsewhere, the seasonality of marriage could be one of the important factors in creating the seasonality in births for the Japanese population (15-17). According to an interview of local health staffs, marriage is most frequent in December and January in this region. If the marriage offers an important occasion to start the sexual activity in this population, the corresponding peak in births would fall in the months after October rather than July to August as observed in the present study. Therefore the marriage hypothesis does not seem appropriate for explaining the seasonality in the studied population. Actually, the Vietnamese

government stresses the family planning policy and the family planning program is the most important in health stations of communities and factories. This general atmosphere might interfere between the seasonality in marriage and in the first births.

From a cultural point of view, Vietnam belongs to the east Asian countries like China, Korea and Japan. Especially, the lunar calender system is very important for the Vietnamese society, as it is for the Chinese. Thus, the lunar new year (February) is widely celebrated. Holland has proposed that the lunar new year holidays is an important occasion to have reproductive activities among the Chinese society in Malaysia, and that a part of the seasonality in births among this population could be explained by this hypothesis (11). Theoretically, conception during the lunar new years holiday corresponds to births in November or December. But unfortunately, in the population studied, the peak falls in July to November. Therefore, the lunar new year theory could not be applicable to this community.

In Vietnamese society, there is an interesting belief concerning conception and birth; i.e. to become pregnant and to give birth in the same year is believed to result in a healthier child, because a child has only one "animal" for himself. In Asian tradition, one of 12 animals is allocated to each year, for example, 1992 is the year of the bird, 1993 that of the monkey. The Vietnamese believe that if a child is conceived in one year and born in the next, the friction between the two animals will cause trouble for the child down through the years. In developing countries like Vietnam, where many children die before 5 years of age, this kind of social belief could play an important role in the conception of children. Furthermore, the Vietnamese government requires that parents have only one or two children. If parents have more than 3 children, they might receive social penalties. However, it is common for parents with one or two daughters but no son to continue reproductive activities until they have at least one son. Therefore, it might be reasonable that parents prefer to have the second child in the same year of conception in expecting a preferable result (especially, a healthy male infant, because the male child preference is still strong in a traditional Vietnamese society). In fact, the September and October birth peak observed in the subsequent births correspond to conception around January. This fact could be explained by the above.

According to Phi Von Ba, to have children is a kind of social security for the Vietnamese parents in order to live their reclining life with ease (24). Of course, to have a healthy baby is a natural desire of parents, but this kind of socio-cultural factor might play some role in the Vietnamese society regarding their behavior concerning reproduction. To evaluate the validity of this theory, further studies should be made in the Vietnamese society.

On the other hand, a number of studies made in tropical or subtropical Asian countries have been concerned with the relation between births and meteorological factors, especially temperature. Chang et al have proposed that a hot and humid summer might inhibit a successful conception because of the temperature effect on genital tissues and/or sexual behavior (5). A number of past reports have supported this meteorological theory (3-5, 26, 28). Table 4 presents the mean temperature and the precipitation in Hochiminh. The hottest and most humid part is from June to September. If the frequency of conception is decreased at this time, as Chang et al suggested, the corresponding births in April, to July would also decrease. In fact, the decrease during these three months is observed in the present data. This result seems to support the meteorological hypothesis, but it does not clarify whether it directly affects the genital tissues or indirectly affects conception through the decrease of coition because of high temperature and humidity. Furthermore, this theory cannot explain the difference in timing of trough and peak between the first and the subsequent births observed in the present study.

Perhaps, one factor alone can not explain this complicated phenomenon, and each of these theories has its proper place in explaining the seasonality of births.

After the introduction of the open market policy, the Vietnamese society is changing very rapidly. Therefore, it will be very interesting to observe if the seasonal variation in births will change according to the social

Table 4 Monthly mean temperature and mean precipitation in Vietnam (Hochiminh city)

	Jun	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean temperature (°C)	25	26	27	28	28	27	26	27	26	26	26	26
Mean Precipitation (mm)	16	3	13	42	220	331	314	269	336	269	115	56

development of the society. If this happens, it will give us more useful information to construct an acceptable explanation for this interesting phenomenon. A number of studies in this field are expected to be realized in the Vietnamese society.

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A NEW BLACKFLY SPECIES OF *SIMULIUM* (*GOMPHOSTILBIA*) FROM SOLOMON ISLANDS, SOUTH PACIFIC (DIPTERA: SIMULIIDAE)

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Abstract: A new blackfly species, *Simulium* (*Gomphostilbia*) *hiroshii* sp. nov. is described based on female, male, pupal and larval specimens collected from the Solomon Islands, South Pacific. This species possesses several characters which are unusual for the subgenus *Gomphostilbia* as follows: in adults, basal part of the radius fully haired in the female but bare in the male, female frons almost bare and shiny, female tergites 5-8 shiny, male genitalia lacking parameral hooks; in the pupal stage, 4 respiratory filaments per side, sterna 6 and 7 devoid of outer hook on each side; larval hypostomium with serrate lateral margins, and larval body with a large conical protuberance dorsomedially on abdominal segment 7, as well as paired dorsolateral small protuberances on abdominal segments 1-4 and metathoracic segment. This is a first record of the subgenus *Gomphostilbia* from the Solomon Islands and represents the most easterly geographical distribution of the subgenus.

There is no taxonomic work on the Simuliidae (Diptera) in the Solomon Islands, South Pacific except that of Stone and Maffi (1971) who reported two species of the subgenus *Morops* Enderlein (i.e., *S.* (*M.*) *sherwoodi* Stone and Maffi, and *S.* (*M.*) sp. nr. *avilae* Smart and Clifford) from Guadalcanal Island. Recently I received larvae, pupae and adults of blackflies collected from the Solomon Islands in 1992 and 1993 by Dr. H. Suzuki. Examination of these samples shows that there are at least seven taxa, of which one belongs to the subgenus *Gomphostilbia* Enderlein. In this paper, I describe this as a new species based on adult female and male specimens caught by a light trap at Honiara, Guadalcanal, where immature stages of this species are not available yet. The association with a pupa and larvae collected from New Georgia Island is tentative and should be ascertained in the future, although the pharate female dissected from the pupa agrees well with the adult females collected from Guadalcanal.

DESCRIPTION

Simulium (*Gomphostilbia*) *hiroshii* sp. nov.

Female. Body length ca. 2.0 mm. *Head.* Narrower than width of thorax. Frons (Fig. 1) black, shiny, with several short hairs along lateral margins; frontal ratio (width at top of eyes and that just above antenna to length) 1.9:1.0:3.4; frons-head ratio (width of frons at top of eyes to maximal width of head) 1.0:5.4. Clypeus black, grey pruinose, with scattered hairs. Fronto-ocular area (Fig. 2) elongate. Antenna composed of 2 + 9 segments, yellow on scape, pedicel and base of 1st flagellar segment, and brown to dark brown on rest parts of flagellar segments (in some specimens yellow up to 3rd or 4th flagellar segment). Maxillary palp brownish black, composed of 5 segments with proportional length of 3rd, 4th and 5th segments 1.0:1.2:2.8; 3rd segment (Fig. 3) of moderate size; sensory vesicle (Figs. 3 & 4) somewhat enlarged, elliptical, with rugged surface, ca. 1/2 × length of 3rd segment, with medium (or large in some palps) round opening little distad to center. Maxillary lacinia with 9 or 10 inner and 13 or 14 outer teenth. Mandible with ca. 18 inner and 12 outer teeth. Cibarium (Fig. 6) with low and wide projection medially along posterior margin, without tubercles. *Thorax.* Scutum yellowish brwon to brown, shiny, not patterned, moderately covered with recumbent, yellow pubescence, interspersed with long, upstanding dark hairs on prescutellar area. Scutellum yellowish brown to brown, with long dark hairs and yellow pubescence. Postscutellum dark brown, grey pruinose, without hairs. Pleural membrane bare. Katepisternum longer than deep, with ca. 14 hairs on each side. *Legs* (Figs. 7-9). All coxae, trochanters, and femora yellow except part of inside surface of mid

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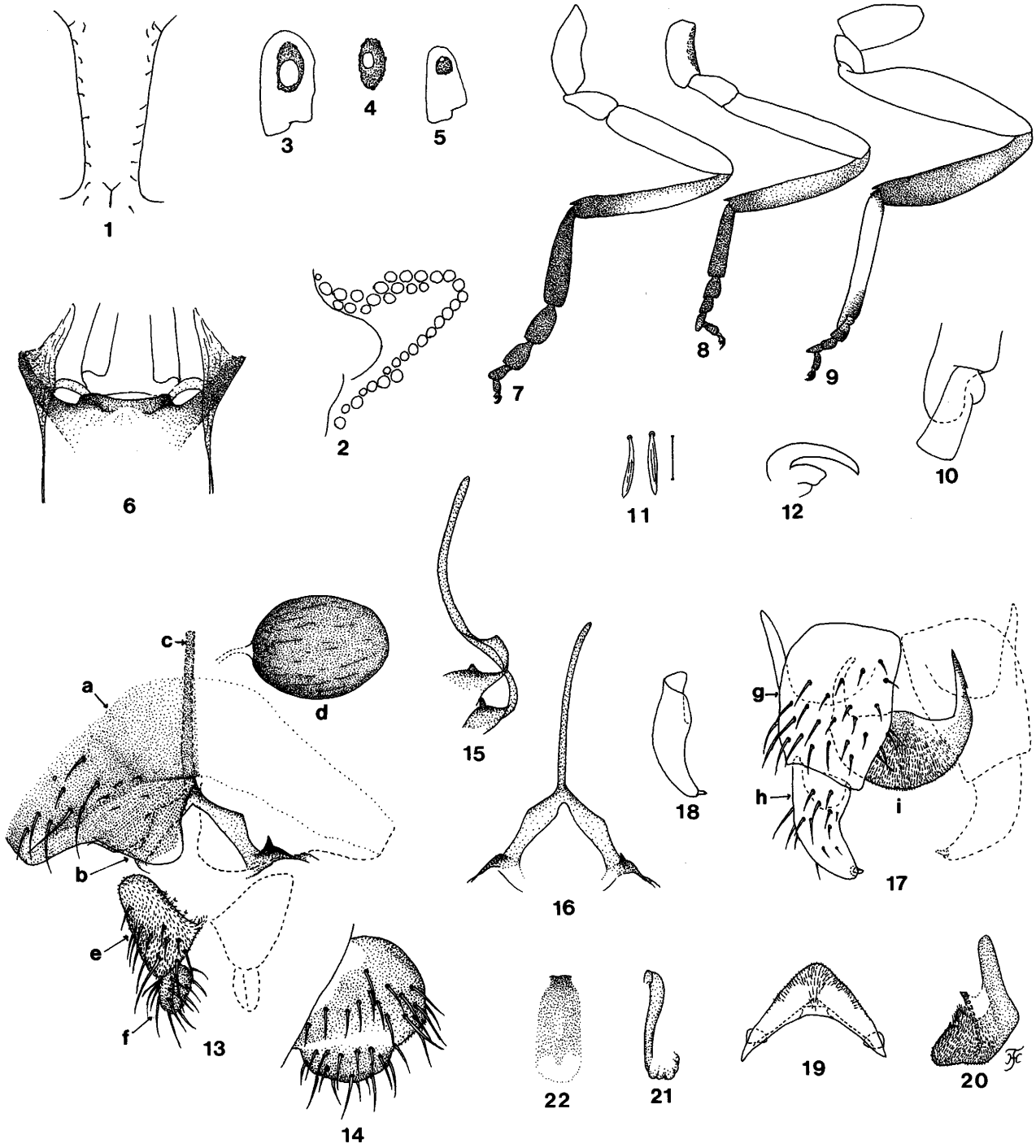
coxa blackish brown. Fore tibia yellow largely in middle, brown along posterior margin, and brownish black on distal 1/4; mid tibia brown with median portion largely pale; hind tibia brown with outer median portion largely somewhat paler; outer surface of fore tibia and posterior surface of mid and hind tibiae largely whitish sheeny when illuminated; outer surface of all femora and tibiae furnished with scale-like setae (Fig. 11). All tarsi brownish black except basal 3/4 of hind basitarsus whitish, and basal 1/2 of hind 2nd tarsomere pale brown. Fore basitarsus somewhat dilated, ca. 4.1 × as long as wide. Hind basitarsus parallel-sided. Calcipala and pedisulcus (Fig. 10) well developed. Tarsal claws (Fig. 12) each with large basal tooth. *Wing.* Length ca. 1.5 mm; costa with spinules and hairs; subcosta haired; basal section of vein R fully haired; hairs at base of stem vein dark brown; basal cell absent. *Abdomen.* Basal scale yellow with fringe of yellow hairs; 2nd segment yellow, widely shiny and whitish iridescent when illuminated; dorsal surface of segments 3 and 4 dark brown, dull, narrowly yellow along anterior margin; tergites 5-8 large, brownish black to black, shining, with dark hairs. *Genitalia* (Figs. 13-16). Sternite 8 (Fig. 13a) well sclerotized, bare medially, with 12-18 short and long stout hairs laterally on each side; anterior gonapophysis (Fig. 13b) triangular in shape, rounded posterointernally, membraneous, covered with a few minute setae as well as numerous microsetae except narrow portion near posterointernal corner bare; inner border slightly curved, narrowly sclerotized. Genital fork (Figs. 13c & 15) of inverted-Y form, with well sclerotized stem; arms somewhat strongly sclerotized along anterior margin on basal 1/2, each with strongly sclerotized distal ridge bearing small to medium projection directed anterodorsally (in some specimens including pharate ♀ this anterodorsal projection absent or very small if any as shown in Fig. 16). Paraproct (Figs. 13e & 14) slightly shorter than wide, with ventral margin rounded, covered with ca. 16 short stout hairs in lateral view. Cercus (Fig. 14) rounded posteriorly, covered with numerous short hairs. Spermatheca (Fig. 13d) ovoid in shape, well sclerotized, with no definite reticulate pattern, with minute internal setae; tube and small area of spermatheca unsclerotized.

Male. Body length ca. 2.0 mm. *Head.* Somewhat wider than thorax. Upper eye consisting of large facets in 14 horizontal rows and in 12 or 13 vertical columns. Clypeus black, thinly grey pruinose, sparsely covered with dark brown hairs. Antenna composed of 2 + 9 segments, coloration as in ♀; 1st flagellomere somewhat elongated, ca. 1.5 × as long as 2nd flagellomere. Maxillary palp composed of 5 segments with proportional length of 3rd, 4th and 5th segments 1.0 : 1.5 : 3.0; 3rd segment (Fig. 5) of normal size with small, globose sensory vesicle being 0.22 × length of 3rd segment; small rounded opening situated basally. *Thorax.* As in ♀. *Legs.* Shape and coloration as in ♀ except as follows: fore basitarsus somewhat dilated, ca. 5.2 × as long as wide; tarsal claws of different form as usual. *Wing.* Length 1.5 mm; other features as in ♀ except subcosta and basal portion of vein R bare. *Abdomen.* Basal scale dark yellow with fringe of long yellow hairs. Dorsal surface of segment 2 yellow, broadly shiny when illuminated; those of segments 3 and 4 dark brown, with anterior margin of segment 3 yellow; tergites of remaining segments large, brownish black, with dark hairs; when illuminated, tergites 5 and 6 with pair of large dorsolateral shiny areas, tergite 7 almost entirely shiny dorsally and laterally except posterodorsal small area dull in middle, tergite 8 with pair of lateral shiny areas. *Genitalia* (Figs. 17-22). Coxite in ventral view (Fig. 17g) ca. 1.5 × as long as wide. Style (Fig. 17h) short, ca. 3/4 × length of coxite, curved inwards, gradually tapered apically, with terminal spine. Ventral plate (Fig. 17i) transverse, rounded posteriorly in ventral view, moderately setose on ventral and posterior surface, with arms somewhat converged; ventral plate much produced ventrally as seen in Figs. 19 & 20. Paramere (Fig. 21) small and slender, lacking parameral hooks. Median sclerite (Fig. 22) broad, plate-like, moderately sclerotized except distal portion transparent.

Pupa. Body length (excluding gill filaments) 2.5 mm. *Head* and *thorax.* Integument yellow, densely covered with tubercles. Head trichomes 4 pairs, all long and simple. Thoracic trichomes 6 pairs (3 anterodorsally, 2 anterolaterally, 1 posterolaterally), all long and simple. Gill (Fig. 23) with 4 slender filaments (length 3.0-3.5 mm) in 2 pairs, short-stalked; 1 of dorsal paired filaments directed upward and outward, other filament directed somewhat upward and forward, and 1 of ventral paired filaments directed outward and forward, then curved forward and somewhat inward, other filament directed downward and forward; all filaments with numerous transverse ridges becoming indistinct toward apical tip, densely covered with minute tubercles (relatively larger tubercles near base). *Abdomen.* All terga transparent or pale yellow, except tergum 9 dark yellow; terga 1 and 2 without tubercles; tergum 1 with single long seta on each side, tergum 2 with 6 simple



Figs. 1-22. Female and male of *Simulium* (*Gomphostilbia*) *hiroshii* sp. nov. 1, female frons; 2, fronto-ocular area; 3, 3rd segment of female maxillary palp showing sensory vesicle with large opening; 4, female sensory vesicle with medium opening; 5, 3rd segment of male maxillary palp; 6, cibarium; 7, female fore leg; 8, female mid leg; 9 female hind leg; 10, distal part of hind basitarsus, and 2nd tarsomere showing calcipala and pedisulcus; 11, scale-like setae on outer surface of femora and tibiae (scale bar 0.02 mm); 12, female claw; 13, female genitalia in situ (ventral view)—a, 8th sternite; b, anterior gonapophysis; c, genital fork; d, spermatheca; e, paraproct; f, cercus; 14, paraproct and cercus (lateral view); 15, genital fork (lateral view); 16, genital fork (ventral view) of pharate female showing a small projection only left side; 17, male genitalia in situ (ventral view)—g, coxite; h, style; i, ventral plate; 18, style (inside view); 19, ventral plate (end view); 20, ventral plate (side view); 21, paramere; 22, median sclerite.

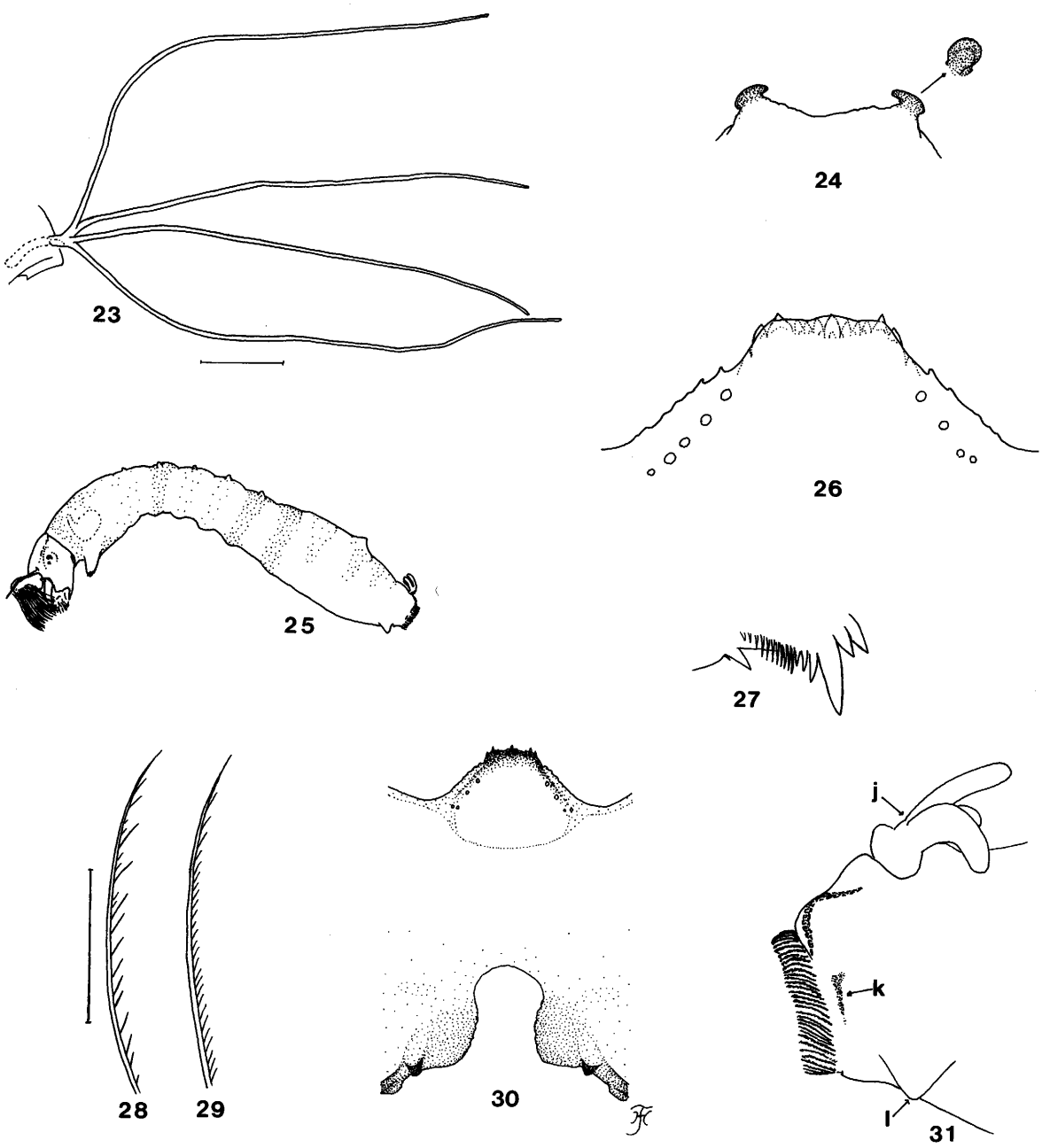


Fig. 23-31. Pupa and larva of *Simulium* (*Gomphostilbia*) *hiroshii* sp. nov. 23, pupal respiratory filaments (side view, scale bar 0.5 mm); 24, pupal terminal hooks (end view), an arrow showing terminal hook viewed posterodorsally; 25, mature larva (side view) showing dorsal protuberances; 26, hypostomium (ventral view); 27, distal tip of larval mandible; 28, innermost main ray of labral fan (scale bar 0.1 mm); 29, one of other main rays of labral fan; 30, head capsule (ventral view) showing a small postgenal cleft; 31, posterior tip of larval abdomen (side view)—j, rectal papilla; k, accessory sclerite; l, ventral papilla.

setae on each side, 1 seta much longer than others. Terga 3 and 4 each with 4 hooked spines directed forward along posterior margin on each side. Terga 6-9 each with spine-combs in transverse row (those on 6th tergum 1 or 2 in number, and smaller, together with spine-combs on 9th tergum, than those on terga 7 and 8) and comb-like groups of minute spines on each side. Tergum 5 devoid of spine-comb. Tergum 9 with pair of round, flat terminal hooks which are constricted at base like rice scoop (Fig. 24). Sternum 4 with a few minute setae on each side; sternum 5 with pair of bifid or trifid hooks on each side; sterna 6 and 7 each with bifid hook, lacking outer hook on each side; grapnel-like hooklets absent on each side of last segment. *Cocoon.* Simple, wall-pocket-shaped, moderately woven, not extending ventrolaterally, floor formed on posterior 2/3.

Mature larva. Body length 4.2-4.4 mm. Body pale reddish purple, with anterior 2/3 of thorax, abdominal segments 1, 4 and 5 entirely greyish, abdominal segments 6, 7 and 8 greyish laterally. Cephalic apotome somewhat darkened, with faint positive head spots. Antenna with 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 3 segments from base to tip 1.0 : 0.6 : 1.1. Labral fan with 37-39 main rays; microtrichia of innermost main ray (Fig. 28) somewhat long, widely spaced, interspersed with 2 smaller microtrichia on distal portion, those of other rays (Fig. 29) short, closely arranged. Mandible (Fig. 27) with comb teeth decreasing in size from 1st to 3rd teeth; mandibular teeth composed of 1 large tooth and 1 small one, without supernumerary serrations. Hypostomium (Fig. 26) with row of 9 apical teeth; median tooth as long as each corner tooth, longer than 3 intermediate teeth on each side; lateral margin serrate anteriorly, undulate posteriorly; hypostomal setae 4 or 5 in number lying subparallel to lateral margins. Postgenal cleft (Fig. 30) small, ca. 4/5 × as long as postgenal bridge. Thoracic cuticle bare. Abdominal cuticle sparsely or moderately covered with colorless, simple and branched (bifid or trifid) minute setae dorsally on posterior segments; sides of anal sclerite of last segment covered with colorless setae. Metathoracic segment and abdominal segments 1-4 each with dorsolateral pair of small conical protuberances; abdominal segment 7 with large conical protuberance dorsomedially (Fig. 25). Rectal papilla (Fig. 31j) of 3 simple lobes. Anal sclerite of usual X-form, with anterior arms slightly shorter than posterior ones, broadly sclerotized at base. Accessory sclerite (Fig. 31k) present. Ventral papillae (Fig. 31l) well developed. Posterior circlet with ca. 80 rows of up to 14 hooklets per row.

Type specimens. Holotype ♀, slide mounted, light-trapped, Mendana Hotel, Honiara, Guadalcanal Island, Solomon Islands, 26. VIII. 1993, H. Suzuki & W. Takayama. Allotype ♂, same data as holotype except date: 21. VIII. 1993. Paratypes 22 ♀, 3 ♂, in alcohol, same data as holotype except date: 15-27. VIII. 1993; 1 pharate ♀, dissected from pupa, 4 mature larvae at Noro, New Georgia Island, New Georgia Islands, Western Province, Solomon Islands, 5. IX. 1992, H. Suzuki.

Holotype, allotype and some paratypes will be deposited at the Natural History Museum, London, U.K, and other paratypes will be deposited at Bishop Museum, Honolulu, U.S.A., and at the Carnegie Museum Natural History, Pittsburgh, U.S.A.

Ecological notes. Adult females and males were caught by a light trap set at Mendana Hotel in Honiara. Aquatic breeding habitats of this species at or near Honiara remain uninvestigated. In New Georgia Island, the pupa and larvae were collected from trailing grasses and dead leaves in a small, fast-flowing stream with width of 1-2 m, running in a natural forest, midpoint between Munda and Noro, together with three species of subgenus *Morops* including *S.* (*M.*) *sherwoodi* Stone and Maffi.

Etymology. This new species was named after Dr. Hiroshi Suzuki, eminent medical zoologist and naturalist, in honor of his winning the 1994 year's prize of Japanese Society of Sanitary Zoology for excellence in his studies on trombiculid mites.

Remarks. *Simulium* (*G.*) *hiroshii* is assigned to the subgenus *Gomphostilbia* by having the haired katepisternum and bare pleural membrane of both sexes of adults. However, it is striking that this species possesses several characters which depart from the diagnoses of the subgenus, redefined by Takaoka and Davies (1994) but agree with those of the other related subgenera.

In the adults, the basal part of the radius is haired in the female but bare in the male. Such sexual dimorphism is of very rare occurrence in the Simuliidae, and has been known to exist in several species of the three Oriental species-groups of the subgenus *Simulium* s. str. (*striatum*-, *multistriatum*-, and *eximium*-groups) (Takaoka and Davies, 1994), and also in some species of the Neotropical subgenus *Psilopelmia.* The female frons of this species is shiny and almost bare, as found in the

subgenus *Simulium* s. str. Most *Gomphostilbia* species have frons dull and fully haired. The female abdomen is shiny only on terga 6-8 in most *Gomphostilbia* species but is also shiny on tergum 5 in this species. Such character has been reported to occur in some species of the *melanopus*-group of the subgenus *Simulium* s. str. (Takaoka, 1983) and in one *Morops* species (Smart and Clifford, 1965). Apart from the bare basal section of the radius, the male of this species exhibits another unusual character in the genitalia in which parameral hooks are absent. The parameral hooks are also undeveloped in all species of the subgenus *Morops* in New Guinea and Australia (Smart and Clifford, 1965; Crosskey, 1967) but are developed in species of the same subgenus in the Philippines and in the Solomon Islands (Takaoka, 1983; Stone and Maffi, 1971).

In the pupal stage, reduced number of respiratory filaments (i.e., 4) and sterna 6 and 7 lacking an outer hook on each side are unusual for *Gomphostilbia* species, of which respiratory filaments are mostly 8 (although rarely 6, 9 and 10), and sterna 6 and 7 bear paired hooks on each side. Recently, one new *Gomphostilbia* species with 4 pupal gill filaments per side was collected from Sulawesi Island, Indonesia, but this belongs to the *ceylonicum*-group which is characterized by the male enlarged hind basitarsus (unpublished data). The unusual character observed on the sterna 6 and 7 is one of the diagnostic characters of the subgenus *Wallacellum* Takaoka in the Philippines (Takaoka, 1983), and is shared by some species of the subgenus *Simulium* s. str. in Central America (Dalmat, 1955). Another pupal character appearing unique to *Gomphostilbia* is found in the shape of terminal hooks.

In larva, most characters of this species generally agree with diagnosic characters of *Gomphostilbia.* However, there are still some characters differing from those of most *Gomphostilbia* species. The pectination of the innermost main ray of labral fans differs from those of other main rays. Similar arrangement of ray microtrichia has been reported at least in *Wallacellum* species. In addition, serrate lateral margins of the hypostomium, which are not unusual in the Simuliidae, are atypical in *Gomphostilbia*, which always bears smooth lateral margins. The paired dorsolateral protuberances of some abdominal segments found in this species have been known in *S.* (*G.*) *parahiyangum* from Java (Takaoka and Sigit, 1992), and in sevral species of different subgenera. However this species seems very unique in having a single large dorsal protuberance on the larval abdominal segment 7, as well as paired ones on anterior segments.

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