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TAXONOMIC NOTES ON *SIMULIUM GOMBAKENSE* (DIPTERA: SIMULIIDAE) FROM PENINSULAR MALAYSIA: DESCRIPTIONS OF MALE AND PUPA, AND SUBGENERIC TRANSFER FROM *MOROPS* TO *GOMPHOSTILBIA*

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Received 1 November, 1999/Accepted 13 December, 1999

Abstract: The male and pupa of *Simulium gombakense* Takaoka and Davies, 1995, hitherto known only from pharate pupal and larval specimens collected from Peninsular Malaysia, are described for the first time. This species is characterized by its pupal gill of much inflated form with six finger-like projections and with eight slender thread-like filaments, similar to those found in some Philippine species of the subgenus *Morops*, in which this species was previously placed. However, *S. gombakense* is here transferred to the subgenus *Gomphostilbia* because many hairs are present on the katepisternum but absent on the pleural membrane in the adult male.

Key words: Simuliidae, black fly, *Gomphostilbia, Morops*, Malaysia, change of subgeneric status, description

Simulium gombakense was described from the pharate pupa and mature larvae collected from Peninsular Malaysia, and was tentatively placed in the subgenus *Morops* Enderlein within the genus *Simulium* Latreille s. 1. (Takaoka and Davies, 1995). However, the adult female and male, and the pupa of this species remained unknown.

In 1996, one pupa and several larvae of *S. gombakense* were collected by the author from the Fraser's Hill, in Peninsular Malaysia, and the adult male was successfully reared from the pupa.

The descriptions of the male and pupa of *S. gombakense* are given for the first time, and based upon the adult male characters, this species is transferred to the subgenus *Gomphostilbia* Enderlein.

***Simulium* (*Gomphostilbia*) *gombakense* Takaoka and Davies, 1995**

Simulium (*Morops*) *gombakense* Takaoka and Davies, 1995: 82–84; Crosskey and Howard, 1997: 45.

DESCRIPTION.

Male. Body length 2.4 mm. ***Head***. Much wider than thorax. Holoptic, upper eye consisting of large facets in 15 vertical columns and in 15 or 16 horizontal rows. Face medium brown, white-pruinose, bare. Clypeus medium to dark brown, white-pruinose, densely covered with golden yellow, scale-like, short hairs, interspersed with dark longer hairs. Antenna composed of 2+9 segments (Fig. 1) (left antenna abnormal, being incompletely segmented between flagellar segments 4 and 5, and also between flagellar segments 8 and 9—Fig. 2), pale yellow except apical 4 flagellar segments light to medium brown; 1st flagellar segment somewhat elongate, ca. 1.5× as long as 2nd flagellar segment. Maxillary palp composed of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.2:3.1; 3rd segment (Figs. 3 and 4) somewhat enlarged; sensory vesicle globular or somewhat ellipsoidal, ca. 0.2× as long as 3rd segment, with small or medium opening apically. ***Thorax***. Scutum medium brown, shiny when illuminated, densely covered with golden-yellow, scale- like, recumbent hairs intermixed with dark similar hairs near anterior margin, and with several dark upright hairs on prescutellar area. Scutellum medium brown, with golden-yellow short hairs as well as dark upright long hairs. Postscutellum medium brown, shiny when illuminated, and bare. Pleural membrane bare. Katepisternum longer than deep, with dark hairs. ***Legs***. Foreleg: coxa whitish yellow; trochanter dark yellow or light brown; femur light brown; tibia light brown with apical 2/5 medium brown, and outer surface of basal 3/5 paler; tibia covered with yellow scale-like hairs on outer surface of basal 3/4, which are bright shiny when il-

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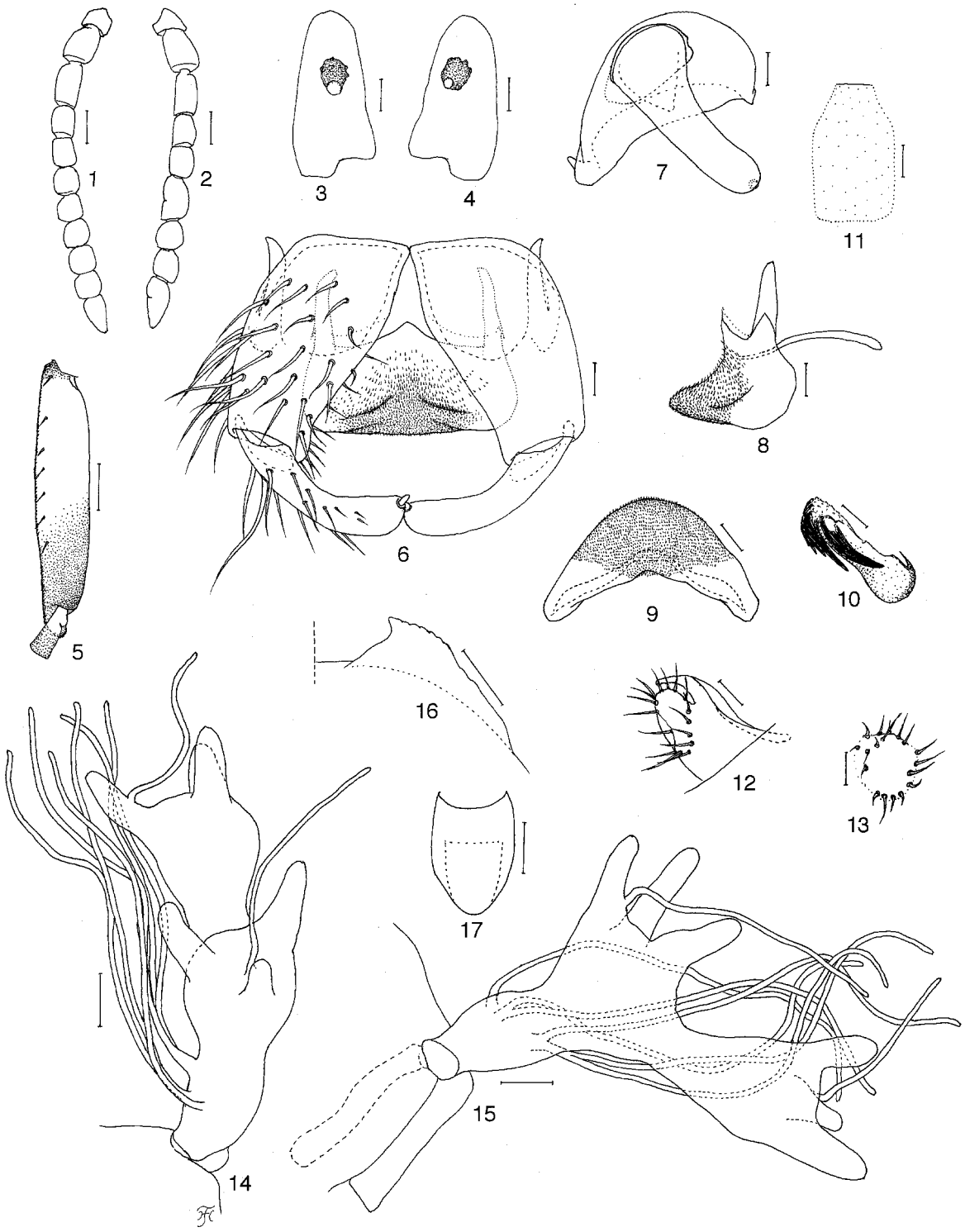
luminated; tarsus medium brown, with moderate dorsal hair-crest; basitarsus slender, ca. 6.8× as long as its greatest width. Midleg: coxa medium brown; trochanter whitish yellow with outer surface light brown; femur medium brown; tibia medium brown with basal 1/4 whitish yellow; basitarsus medium brown with basal 1/3 or more slightly paler, other tarsal segments medium brown. Hind leg: coxa light brown; trochanter pale yellow; femur light brown with apical cap dark brown and basal tip pale yellow; tibia light to medium brown except basal 1/2 whitish yellow; tarsus light brown except basal 1/2 of basitarsus and of 2nd segment yellowish white; basitarus (Fig. 5) somewhat enlarged, nearly parallel-sided on basal 2/3 but gradually narrowed on apical 1/3, ca. 5.0× as long as its greatest width, and ca. 0.7× as wide as tibia, which is subequal in greatest width to femur; calcipala moderately developed, ca. 1.2× as long as wide, and pedisulcus moderately developed. All femora, tibiae and parts of tarsus covered with scale-like hairs as well as usual simple hairs on outer surface. ***Wing***. Length 1.9 mm; costa with spines and hairs; subcosta bare; basal portion of radial vein fully haired; tuft hair of base of costa and base of radial vein dark but brightly golden yellow when illuminated; basal cell absent. ***Abdomen***. Basal scale dark brown with fringe of dark long hairs laterally. Dorsal surface of abdomen medium to dark brown except basal 1/2 of 2nd segment whitish, moderately covered with dark short hairs; segments 2, 5, 6, 7 and 8 each with a pair of shiny (often iridescent) dorsolateral or lateral patches, those on segment 2 connected in middle to each other. ***Genitalia***. Coxite (Fig. 6) enlarged, much longer than wide. Style (Figs. 6 and 7) slender, 0.87× as long as coxite, nearly parallel-sided when viewed ventrally, somewhat curved inwardly, with 1 apical spine. Ventral plate in ventral view (Fig. 6) transverse, much wider than long, somewhat diverged posteriorly, widest just before posterior margin, moderately covered with microsetae on ventral surface, with posterior margin nearly straight, but anterior margin convex medially; basal arms nearly parallel-sided, and somewhat curved inwards; ventral plate in lateral view (Fig. 8) prominently produced ventrally and also produced dorsally, though to less extent, near posterior margin; ventral plate in end view (Fig. 9) round ventrally, and densely covered with microsetae on most medial portion of posterior surface. Parameres (Fig. 10) of moderate size, each with 2 long stout hooks and a few medium or short ones. Median sclerite (Fig. 11) thin and broad, plate-like. Cercus (Figs. 12 and 13) small, encircled with 14-18 short hairs. Aedeagal membrane densely covered with microsetae.

Pupa. Body length ca. 2.8. mm. ***Head***. Integument yellow, moderately covered with round tubercles (only on frons); antennal sheath bare, and without any projections; face with 1 pair of trifid, long trichomes, and frons with 3 pairs of bifid or trifid, long trichomes; all trichomes subequal in length and stoutness to one another. ***Thorax***. Integument yellow, moderately covered with round tubercles, with 3 pairs of bifid, long trichomes dorsally, with 2 pairs of bifid, long trichomes anterolaterally, with 1 pair of bifid, medium trichomes posterolaterally, and with 3 pairs of simple or bifid trichomes, of which 2 are medium and 1 is short, ventrolaterally. Gill (Figs. 14 and 15) of much inflated structure (ca. 0.9 mm long), with 2 triplet groups of short, finger-like projections (length 0.2 mm or a little less), 1 group directed upwards and the other directed forwards, with 8 slender thread-like filaments (in the original description, 2 shorter filaments were overlooked); 6 filaments arise near base from inside surface of inflated structure, arranged in 1+2+3 filaments from dorsal to ventral, and subequal in length (ca. 0.8 mm) and thickness to one another, while remaining 2 arise individually from dorsal surface of triplet groups, of which 1 filament (ca. 0.5 mm long) arises from base of dorsalmost finger-like projection or near it in dorsal triplet group, and the other (ca. 0.3 mm long) from base of innermost finger-like projection in anterior triplet group, these 2 isolated filaments subequal in thickness to the other ones; surface of inflated portion and all filaments very lightly greyish yellow-colored, without any pattern (on inflated portion) or any transverse ridges or furrows (on filaments), and densely covered with minute tubercles. ***Abdomen***. Tergum 1 pale, bare, with 1 simple or bifid, long hair on each side; tergum 2 with 1 simple long hair and 5 very minute simple setae on each side; terga 3 and 4 each with 4 hooked spines and 1 minute seta on each side; tergum 5 lacking spine-combs and comb-like groups of minute spines; terga 6-8 each with spine-combs and comb-like groups of minute spines in transverse row on each side; tergum 9 with comb-like groups of minute spines in transverse row, and a pair of distinct plate-like terminal hooks (Fig. 16). Sternum 4 with 1 simple hook and 1 minute seta on each side; sternum 5 with a pair of bifid hooks submedially and a few minute setae on each side; sterna 6 and 7 each with a pair of bifid inner and simple outer hooks somewhat spaced from each other, and a few minute setae on each side; 3 grapnel-like hooklets on each side of segment 9. ***Cocoon*** (Fig. 17). Wall-pocket-shaped, thinly and com-



Figures 1–17. Male and pupal characters of *Simulium gombakense*. 1–13, male; 14–17, pupa. 1 and 2, right and left antennae (lateral view); 3 and 4, right and left 3rd maxillary palpal segments with sensory vesicle (front view); 5, left hind basitarsus and 2nd tarsal segment (outside view); 6, coxites, styles and ventral plate *in situ* (ventral view); 7, right coxite and style (end view); 8, ventral plate and median sclerite (lateral view); 9, vental plate (end view); 10, left paramere (end view); 11, median sclerite (end view); 12 and 13, left cerci (12, lateral view and 13, end view); 14 and 15, right gills (14, dorsal view; 15, lateral view); 16, left terminal hook (dorsal view); 17, cocoon (dorsal view). Scale bars 1.0 mm for fig. 17; 0.1 mm for figs. 5, 14 and 15; 0.05 mm for figs. 1 and 2; 0.02 mm for figs. 3, 4, 6–13 and 16.

pactly woven with no open spaces in webs, somewhat extending ventrolaterally, covering entire abdomen and posterior 1/2 of thorax; anterior margin not thickened; floor loosely woven only on posterior 1/2; individual threads invisible; ca. 2.3 mm long ×1.7 mm wide.

SPECIMENS EXAMINED. 1 male adult reared from pupa, and its associated pupal skin and cocoon, collected from a small stream, near Gap, Fraser's Hill, Pahang, Peninsular Malaysia, 20. III. 1996, by A. Takaoka and H. Takaoka.

ECOLOGICAL NOTES. The pupa and several larvae of this species were collected on trailing grass leaves in a small, shaded stream with its width of 0.3–0.6 m and water temperature of 22.0°C. Altitude was ca. 700 m. Five other blackfly species, i. e., *S. asakoae, S. bishopi, S. malayense, S. tani* and *S. whartoni,* were also collected from the same stream.

DISTRIBUTION. Peninsular Malaysia and Thailand (Dr. C. Kuvangkadilok, unpublished data).

REMARKS. *Simulium gombakense* was described as a new species from the one pharate pupa and four mature larvae collected from a tributary of Gombak River, and from a tributary of Batu River in Peninsular Malaysia (Takaoka and Davies, 1995). This species appeared to be assigned to either the subgenus *Gomphostilbia* or the Philippine group of subgenus *Morops* (Takaoka, 1983), by having the plate-like terminal hooks, the grapnel-like hooklets on the last abdominal segment, in the pupa, and the smooth lateral margins of the hypostomium, and the presence of the ventral papillae on the last abdominal segment, in the larva. However, this species was tentatively placed to the subgenus *Morops* because of its form of the pupal gill being much inflated with slender filaments, as shown in Figs. 15 and 16. The similar form of the pupal gill had been known in two Philippine species (i. e., *S. ifugaoense* and *S. manbucalense*) of the subgenus *Morops* (Takaoka, 1983), but not in the subgenus *Gomphostilbia* until recently when we reported one related new species of *Gomphostilbia* from Sumatra (Takaoka and Sigit, 1997).

Despite of this similarity in the form of the pupal gill between this species and the two Philippine species of *Morops,* the male adult of *S. gombakense* examined in this study clearly shows that it belongs to the subgenus *Gomphostilbia* by having the katepisternum haired but pleural membrane bare, one of the key diagnostic characters of the subgenus *Gomphostilbia* separating it from *Morops,* according to the definition given by Crosskey (1967). In all the known *Morops* species both katepisternum and pleural membrane of the adults are haired (Crosskey, 1967).

Acknowledgements

I am grateful to Dr. Yong Hoi Sen, Professor Emeritus, University of Malaya, for his kind arrangement of my collection trip made in 1996. Thanks are due to Dr. Chaliow Kuvangkadilok, Mahidol University, who gave me an opportunity to examine the Thailand specimens of *S. gombakense.* This study was supported in part by the Japan Society for the Promotion of Science, and also by the Grant-in-Aid of the Ministry of Education, Science, Sports and Culture, Japan (No. 11670246).

References

1) Crosskey, R.W. (1967): The classification of *Simulium* Latreille (Diptera: Simuliidae) from Australia, New Guinea and the Western Pacific. J. Natur. Hist. 1, 23–51
2) Crosskey, R.W. and Howard, T.M. (1997): A New Taxonomic and Geographical Inventory of World Blackflies (Diptera: Simuliidae). 144 pp., The Natur. Hist. Mus., London.
3) Takaoka, H. (1983): The Blackflies (Diptera: Simuliidae) of the Philippines. xi+199 pp., The Japan Society for the Promotion of Science, Tokyo.
4) Takaoka, H. and Davies, D.M. (1995): The Black Flies (Diptera: Simuliidae) of West Malaysia. viii+175 pp., Kyushu University Press, Fukuoka.
5) Takaoka, H. and Sigit, S.H. (1997): Three new black fly species of *Simulium* (Diptera: Simuliidae) from Sumatra, Indonesia. Jpn. J. Trop. Med. Hyg., 25 (2), 69–80

IMMUNOBLOT ANALYSIS OF ANTIGENS FROM *PARASTRONGYLUS CANTONENSIS, P. COSTARICENSIS* AND *P. MALAYSIENSIS* USING SERUM ANTIBODIES AGAINST *P. CANTONENSIS*

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Abstract: *Parastrongylus cantonensis, P. costaricensis* and *P. malaysiensis* adult worm antigens were tested for cross-reactivities in immunoblots using sera from patients with parasitologically confirmed parastrongyliasis. Sera from *P. cantonensis*-infected patients with a high specificity for the 31 kDa diagnostic *P. cantonensis* antigen also cross-reacted with a corresponding component found in *P. costaricensis* and *P. malaysiensis* proteins. The immune sera consistently recognized a prominent immunogenic band with molecular weight of 31 kDa against the antigens prepared from the three *Parastrongylus* species. Sera obtained from other clinically related patients infected with *Gnathostoma spinigerum* and normal healthy control sera did not react with the 31 kDa protein from the three species of *Parastrongylus*. The cross-reactivity observed in the immunoblots performed on serum samples from patients with *P. cantonensis* infection indicates that the three *Parastrongylus* species share the specific 31 kDa antigen. This cross-reacting protein may also be applied for an immunodiagnosis of human infection with the other two species of *Parastrongylus* worms, viz. *P. costaricensis* and *P. malaysiensis*.

Key words: *Parastrongylus* (=*Angiostrongylus*) antigens, Immunoblotting, Immunodiagnosis, *P. cantonensis, P. costaricensis, P. malaysiensis*

INTRODUCTION

Among the 20 species recognized in the genus *Parastrongylus* (syn. *Angiostrongylus*), *P. cantonensis* which is a nutural parasite inhabiting the lung of rats, has been incriminated as a cause of human eosinophilic meningitis in the Asia-Pacific region (Rosen *et al*., 1967; Alicata and Jindrak, 1970; Cross, 1987; Kliks and Palumbo, 1992), while *P. costaricensis* which inhabits the mesenterium of rats has been reported to cause human abdominal granuloma in Central and South America (Morera, 1973, 1985). In addition, *P. malaysiensis* which is closely related to *P. cantonensis* has been reported to be the possible causative agent of human *Parastrongylus*-induced eosinophilic meningitis in Malaysia (Watts, 1969; Bisseru *et al*., 1972; Ambu *et al*., 1994).

Diagnosis of human parastrongyliasis, i.e., *P. cantonensis*, *P. costaricensis* and *P. malaysiensis* infections, is based on clinical, epidemiological, and laboratory criteria. Various immunodiagnostic techniques utilizing partially purified adult worm antigens of *P. cantonensis* have been successfully used for the specific diagnosis of the infection (Kliks *et al*., 1988; Ko, 1989). Nonetheless except for the study by Ambu *et al*. (1997), relatively few studies have been carried out on the specific immunodiagnosis of human infections caused by *P. costaricensis* and *P. malaysiensis*. One reason might be the lack of available antigens. Moreover, it is also difficult to obtain a number of parasitologically confirmed patient sera for use as a gold standard. Since the development and application of immunological tests are usually impaired by difficulty in obtaining sufficient amounts of specific parasite antigens to guarantee the homogeneity and quality control of antigen lots. The possibility of using an alternative source for the preparation of appropriate antigens might be based on the

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observation that different species of *Parastrongylus* worm share common antigens.

A *P. cantonensis*-antigen of 31 kDa which is consistently recognized by sera from *P. cantonensis*-infected patients, but not from uninfected persons or of individuals with other parasitic infections, has recently been described (Akao *et al*., 1992; Eamsobhana, 1994). Due to the high specificity and sensitivity of the reaction, the diagnostic potential for this 31 kDa protein has been proposed (Nuamtanong, 1996; Eamsobhana *et al*., 1997). On the contrary, the antigenic components of *P. costaricensis* and *P. malaysiensis* which induce specific antibody responses have not been well defined. Knowledge of individual antigens, their abundance, and their immunogenicity is needed to develop a specific immunodiagnostic test.

In the present communication, the reactivity of sera from patients infected with *P. cantonensis* was investigated with *P. costaricensis* and *P. malaysiensis* antigens as well as with *P. cantonensis* antigens.

Materials and Methods

Antigen preparation

The Thailand strain of *P. cantonensis* used in this study was provided to us in 1996 by Dr. Chalit Komalamisra, Faculty of Tropical Medicine, Mahidol University, and has been maintained in our laboratory. The Costa Rican strain of *P. costaricensis* worms were kindly provided by Dr. Akira Ishii and Dr. Hideto Kino, Hamamatsu University School of Medicine, Japan. Adult worms of *P. cantonensis* and *P. malaysiensis* were obtained from the pulmonary arteries of experimentally infected albino rats (*Rattus norvegicus*), while adult worms of *P. costaricensis* were obtained from the mesenteric arteries of experimentally infected cotton rats (*Sigmodon hispidus*). The third-stage larvae of *P. cantonensis* and *P. costaricensis* used for infections were collected from the snails, *Biomphalaria glabrata*, that had been infected with first-stage larvae from the feces of infected rats. The infective larvae of *P. malaysiensis* were collected from slugs found in the state of Selangor, Malaysia.

Soluble antigens from *P. cantonensis, P. costaricensis* and *P. malaysiensis* were prepared by standard procedures as previously described (Eamsobhana *et al*., 1997). Briefly, male and female worms of each species were washed and then homogenized separately in a small volume of normal saline solution using a glass tissue grinder. The suspension was then sonicated and kept overnight at 4°C to allow elution of antigens. After centrifugation at 4,000 rpm for 15 min, the supernatant containing soluble antigens was collected and the protein content estimated using Protein Assay Kit II (Bio-Rad Laboratories, USA).

Sera

Serum samples were obtained from five patients with parasitologically confirmed parastrongyliasis (3 with cerebral parastrongyliasis from whom *P. cantonensis* larvae were recovered from the CSF; other 2 had ocular parastrongyliasis from whom immature *P. cantonensis* worms were recovered from their eye chamberes).

Two heterologous sera were collected from patients with confirmed gnathostomiasis (both the cases had *G. spinigerum* larva recovered from the skin). The normal control sera were obtained from 2 healthy adults who were negative for any parasitic infection at the time of blood collection. All serum samples were kept at −20°C until use.

Electrophoresis and immunoblotting

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the procedure previously described (Eamsobhana *et al*., 1997). Briefly, The male and female worm extracts of *P. cantonensis, P. costaricensis* and *P. malaysiensis* (10 μg of proteins per lane) were separated on a 12% reducing SDS-polyacrylamide slab gel using the method of Laemmli (1970). After electrophoresis, the resolved polypeptide bands were either revealed by staining with Coomassie brilliant blue R 250 or electrophoretically transferred from the gel to a nitrocellulose membrane for immunoblotting, which was conducted as described by Towbin *et al*. (1979). After protein transfer, the blotted membrane was treated overnight at 4°C with the test serum. Antigens recognized by serum antibodies were revealed with horseradish peroxidase-conjugated goat anti-human immunoglobulins (Dakopatt, Denmark) using 4-chloro-1-naphthol (Bio-Rad) as substrate. The molecular weight of the separated proteins was estimated by comparing their electrophoretic mobilities with those of known standard molecular weight markers (Sigma Chemical Company, USA).

Results

The polypeptide pattern obtained from adult worm extracts of *P. cantonensis, P. costaricensis* and *P. malaysiensis* as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was

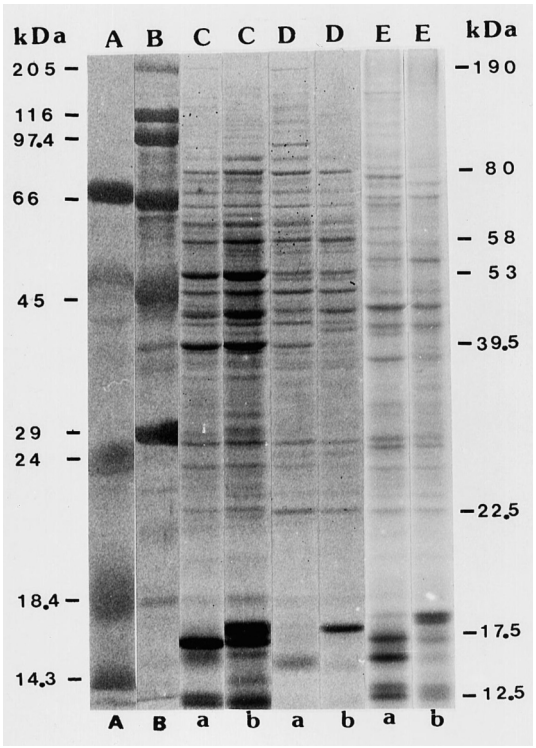

Figure 1 SDS-PAGE profiles of *Parastrongylus cantonensis, P. costaricensis* and *P. malaysiensis* adult worm extracts, stained with Coomassie brilliant blue. C: *P. cantonensis*; D: *P. costaricensis*; E: *P. malaysiensis*; A: low molecular weight markers (Sigma); B: high molecular weight markers (Sigma). (a) Female adult worm extracts. (b) Male adult worm extracts.

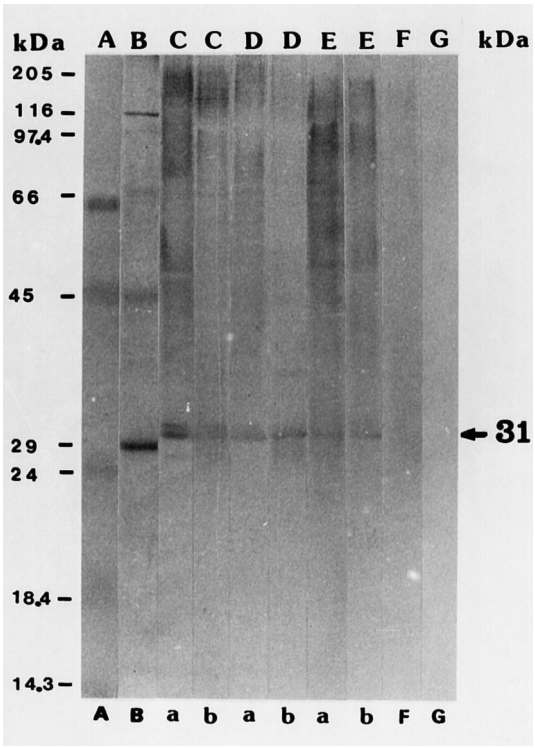

Figure 2 Immunoblot of a representative serum sample from patient infected with *Parastrongylus cantonensis* reacted with antigens of *P. cantonensis* (C), *P. costaricensis* (D) and *P. malaysiensis* (E). Serum of patient infected with *Gnathostoma spinigerum* reacted with *P. costaricensis* antigens (F). Normal healthy control serum reacted with *P. costaricensis* antigens (G). Female adult worm antigens (a). Male adult worm antigens (b). Low and high molecular weight markers (Sigma) (A, B).

quite similar to each other. At least 40 major bands with molecular weight ranging from 12.5 to 205 kDa were observed. However, differences in the intensity of individual peptides existed. The polypeptides with molecular weight of 53, 39.5, 25, 17.5, 14.3 and 12.5 kDa from *P. costaricensis*, and 60, 53, 45, 39.5, 22.5 and 17.5 kDa from *P. malaysiensis* were present only in small or trace quantities. The complexity of the polypeptide patterns is shown in Figure 1.

Immunoperoxidase staining in immunoblots of *P. cantonensis, P. costaricensis* and *P. malaysiensis* antigens against a representative human serum with parasitologically confirmed parastrongyliasis is shown in Figure 2. About 18 immunogenic components of adult worm extracts from *P. cantonensis* were recognized by the sera of patients infected with *P. cantonensis*. The sera reacted strongly with the 31 kDa peptide of both the female and male worm antigens of *P. cantonensis* (Figure C). The pattern of reactivities for *P. costaricensis* and *P. malaysiensis* antigens was similar to that obtained with *P. cantonensis* antigens. Cross-reactions of varying intensity and a varying number of bands were observed with the antigens of *P. costaricensis* and *P. malaysiensis*. These sera also recognized the 31 kDa component among adult *P. costaricensis* and *P. malaysiensis* proteins (Figures D, E). The cross-reactive antigenic components of the three *Parastrongylus* species recognized by sera of patients with gnathostomiasis were variable but the 31 kDa peptide was not recognized. The reactivity obtained with the representative serum of patient infected with *Gnathostoma spinigerum* is shown in Figure F. No prominent band was observed when normal human sera were reacted with *P. cantonensis, P. costaricensis* and *P. malaysiensis* antigens (Figure G).

Discussion

Parasitologic diagnosis of human parastrongyliasis caused by *P. cantonensis, P. costaricensis* and *P. malaysiensis* remains difficult. Recently, a specific immunodiagnostic test for *P. cantonensis* infection based on detection of antibody to a specific antigen with molecular weight of 31 kDa by immunoblotting has been described (Akao *et al*., 1992; Eamsobhana, 1994; Nuamtanong, 1996; Eamsobhana *et al*., 1997). This method is used to determine whether *P. cantonensis* shares specific common antigen with a closely related species, *P. malaysiensis* as well as *P. costaricensis*.

In the present investigation, the protein components of the crude extracts of *P. cantonensis, P. costaricensis* and *P. malaysiensis* and their reactivities with serum antibodies from infected patients were compared by SDS-PAGE and immunoblots. The protein extracts from the three *Parastrongylus* species contained similar as well as different components. However, all three contained an antigen of 31 kDa which has been found to be a specific diagnostic antigen for human infection caused by *P. cantonensis*. Thus the adult worms of any of these three *Parastrongylus* species seem equally suitable for preparing extracts containing this particular antigen for diagnosis.

The present immunoblot confirms that a diagnostic antigen of 31 kDa from *P. cantonensis* has the sensitivity and specificity predicted from our previous studies (Eamsobhana *et al*., 1996). All sera from patients with parasitologically proven parastrongyliasis, contained the specific antibody identified by the 31 kDa antigen of *P. cantonensis*, and this was also detected using extracts of *P. costaricensis* and *P. malaysiensis*. Thus, the 31 kDa common antigen present in these three species of *Parastrongylus* was not only similar in size, but also antigenically related. The 31 kDa antigen of *P. costaricensis* and *P. malaysiensis* cross-reacted consistently with *P. cantonensis* infected patient sera, but never with sera obtained from patients with gnathostomiasis, a clinically related parasitic infection or from uninfected persons. This extends our previous observations on the specificity of the reaction with this antigen for human parastrongyliasis (Eamsobahana, 1994; Eambsohana *et al*., 1997). Since immunoblotting technique using *P. cantonensis* antigens can detect specific antibody in *P. cantonensis* infection, it is considered that *P. cantonensis* antigens may be used for detection of the corresponding antibody in human infections with the other two species of *Parastrongylus, P. costaricensis* and *P. malaysiensis*.

In this study we considered only the diagnostic potential of cross-reactions between *P. costaricensis*/*P. malaysiensis* antigens and *P. cantonensis*-infection sera. Further purification of this cross-reactive 31 kDa protein to study its possible biological functions remains to be established.

Acknowledgment

We are indebted to Dr. Akita Ishii and Dr. Hideto Kino (Hamamatsu University School of Medicine, Japan) for the Costa Rican strain of *P. costaricensis*, to Dr. Chalit Komalamisra for the Thailand strain of *P. cantonensis*, and to Dr. Wanchai Maleewong and Dr. Peera Buranakitjareon for the sera from patients with parastrongyliasis and gnathostomiasis. We also appreciate the photographic assistance rendered by Mr. Somkuan Suvuttho. This study was funded in part by the Siriraj China Medical Board Grant No. 75-348-269 to the senior author.

References

1) Akao, N., Kondo, K., Ohyama, T., Chen, E. and Sano, M. (1992): Antigens of adult female worms of *Angiostrongylus cantonensis* recognized by infected humans. Japanese J. Parasitol., 41, 225-230

2) Alicata, J.E. and Jindrak, K. (1970): Morphology of the adult parasites and larval stages. *In*: Angiostrongyliasis in the Pacific and Southeast Asia. H.H. Anderson (ed.). pp. 8-16. Springfield, Illinois; Charles C Thomas

3) Ambu, S., Noor Rain A., Mak, J.W. and Haidah, S. (1994): Serological survey of *P. malaysiensis* infection in Malaysia. Trend on the management and control of tropical diseases: Proceedings of the First International Congress of Parasitology and Tropical Medicine, Kuala Lumpur, Malaysia

4) Ambu, S., Noor Rain A., Mak, J.W., Maslah, D. and Haidah, S. (1997): Detection of *Angiostrongylus malaysiensis* circulating antigen using monoclonal antibody-based enzyme-linked immunsorbent assay (MAb-ELISA). Southeast Asian J. Trop. Med. Public Health, 28 (Suppl. 1), 143-147

5) Bisseru, B., Gill, S.S. and Lucas, I.K. (1972): Human infections with rat lung worm *Angiostrongylus cantonensis* (Chen, 1935) in West Malaysia. Med. J. Malaya, 2, 164-167

6) Cross, J.H. (1987): Public health importance of *Angiostrongylus cantonensis* and its relative. Parasitol. Today, 3, 367-369

7) Eamsobhana, P. (1994): Immunological studies on the rat lung-worm *Angiostrongylus cantonensis* (Nematoda: Metastrongylidae). pp. 69-100. PhD Thesis, University of Malaya, Kuala Lumpur

8) Eamsobhana, P., Mak, J.W. and Yong, H.S. (1996):

Development of specific immunodiagnosis for human parastrongyliasis. *In*: Proceedings of the 8th SEAMIC/IMFJ Technical Meeting on Molecular Biology and Immunology in the Diagnosis of Parasitic Diseases with emphasis on Malaria. pp. 159–165. SEAMIC Publication, Japan.

9) Eamsobhana, P., Mak, J.W. and Yong, H.S. (1997): Identification of *Parastrongylus cantonensis* specific antigens for use in immunodiagnosis. Int. Med. Res., 1, 1–5

10) Kliks, M.M., Lau, W.K.K. and Palumbo, N.E. (1988): Neurologic angiostrongyliasis: parasitic eosinophilic meningoencephalitis. In Laboratory diagnosis of infectious disease: principles and practices. A. Balows, W.J. Hausler, M. Ohashi, A. Turn (eds.). pp. 754–767. Springer-Verlag, New York.

11) Kliks, M.M. and Palumbo, N.E. (1992): Eosinophilic meningitis beyond the Pacific Basin: the global dispersal of a peridomestic zoonosis caused by *Angiostrongylus cantonensis*, the nematode lungworm of rats. Soc. Sci. Med., 34, 199–212

12) Ko, R.C. (1989): Application of serological techniques for the diagnosis of angiostrongyliasis. *In*: Current Concepts in Parasitology. R.C. Ko (ed). pp. 101–110. The University of Hong Kong Press

13) Laemmli, U.K. (1970): Clevage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 277, 680–685

14) Morera, P. (1973): Life history and redescription of *Angiostrongylus costaricensis* Morera and Cespedes, 1971. Am. J. Trop. Med. Hyg., 22, 613–621

15) Morera, P. (1985): Abdominal angiostrongyliasis: A problem of public health. Parasitol. Today, 1, 173–175

16) Nuamtanong, S. (1996): The evaluation of the 29 and 31 kDa antigens in female *Angiostrongylus cantonensis* for serodiagnosis of human angiostrongyliasis. Southeast Asian J. Trop. Med. Public Health, 27, 291–296

17) Rosen, I., Loison, G., Laigret, J. and Wallace, G.D. (1967): Studies on eosinophilic meningitis. 3. Epidemiologic and clinical observations on Pacific Islands and possible etiologic role of *Angiostrongylus cantonensis*. Am. J. Epidemiol., 85, 17–44

18) Towbin, H., Staehelin, T. and Bordon, L. (1979): Electrophoresis transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA., 76, 4350– 4354

19) Watts, H.B. (1969): Five cases of eosinophilic meningitis in Sarawak. Med. J. Malaya, 24, 89–93

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