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SEASONAL AND DIURNAL BITING ACTIVITIES AND ZOONOTIC FILARIAL INFECTIONS OF TWO *SIMULIUM* SPECIES (DIPTERA: SIMULIIDAE) IN NORTHERN THAILAND

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Summary:

Seasonal and daily biting activity patterns, and natural filarial infections of adult black flies attracted to human bait were investigated at Ban Pang Faen, a rural area in Chiang Mai Province in northern Thailand. Collections were carried out twice a month from 06-00 to 18-00 hours from January 2005 to February 2006. Among ten *Simulium* species collected, *S. nodosum* and *S. asakoae* were predominant occupying 57.3 % and 37.2 % of the total 16,553 females, respectively. These two predominant species showed different patterns in seasonal abundance: majority of *S. nodosum* (86.7 %) were collected in hot season (from mid February to mid May), while most of *S. asakoae* (74.5 %) were collected in rainy season (from mid May to mid October). For the daily biting activity, *S. nodosum* had two patterns: the main one was unimodal with a peak from 17-00 to 18-00, and the other was bimodal and had the major peak from 16-00 to 18-00 and the minor one from 07-00 to 09-00. The pattern of *S. asakoae* was mostly unimodal with a peak from 06-00 to 10-00. The filarial larvae found in *S. nodosum* and *S. asakoae* were morphologically different from each other. The short and thick infective larvae found in *S. asakoae* differed from all known filarial larvae; it is suggested that they might be a bird parasite, Splendidofilariinae or Lemdaninae. The infection of the mammophilic *S. nodosum* with large *Onchocerca* type infective larvae was confirmed in this area. Natural filarial infections were found in each month (except December) in either *S. nodosum* or *S. asakoae* or in both. Monthly infection rates with all stages of larvae were 0.6-5.0 % for *S. nodosum*, and 1.0-4.0 % for *S. asakoae*. It is suggested that people in this village are exposed to the risk of infection with zoonotic filariae throughout the year.

KEY WORDS : black fly, biting activity, filaria, natural infection, Simuliidae, Thailand.

Résumé : ÉTUDE DES RYTHMES JOURNALIERS ET SAISONNIERS D'ACTIVITÉ DE VOL ET DE L'INFECTION NATURELLE DE DEUX ESPÈCES DE SIMULIES (DIPTERA: SIMULIIDAE) DANS LE NORD DE LA THAILANDE

Les rythmes journaliers et saisonniers d'activité de vol et de l'infection naturelle des simulies attirées par appât humain ont été étudiés dans le village de Ban Pang Faen, Province de Chiang Mai, nord de la Thaïlande. Les captures ont été effectuées deux fois par mois de 6 heures à 18 h, de janvier 2005 à février 2006. Parmi les dix espèces de Simulium *récoltées,* S. nodosum *et* S. asakoae *prédominent, représentant respectivement 57.3 % et 37.2 % des 16553 simulies femelles capturées. Ces deux espèces montrent des schémas distincts d'abondance saisonnière : la majorité des* S. nodosum *(86.7 %) a été récoltée à la saison chaude (de mi-février à mi-mai), tandis que la majorité des* S. asakoae *(74.5 %) a été récoltée pendant la saison des pluies (de mi-mai à mi-octobre). Pour l'activité journalière de vol,* S. nodosum *présente deux courbes : la principale est unimodale avec un pic entre 17 h et 18 h; l'autre est bimodale avec un pic principal entre 16 h et 18 h et un pic mineur entre 7 h et 9 h. Chez* S. asakoae*, la courbe est principalement unimodale avec un pic entre 6 h et 10 h. Les larves de filaires récoltées chez* S. nodosum *et* S. asakoae *sont morphologiquement différentes. Les larves infectantes courtes et épaisses récoltées chez* S. asakoae *sont distinctes de toutes celles qui sont connues; il est suggéré qu'elles pourraient être des parasites d'oiseaux, Splendidofilariinae ou Lemdaninae. L'infection de l'espèce mammophile* S. nodosum *par de grandes larves infectantes de type* Onchocerca *est confirmée dans ce village. Les infections naturelles ont été trouvées chaque mois (sauf en décembre) chez* S. nodosum*, ou chez* S. asakoae*, ou chez les deux. Les taux mensuels d'infection calculés avec tous les stades de filaires ont été de 0,6-5,0 % pour* S. nodosum *et 1,0-4,0 % pour* S. asakoae*. Il est suggéré que la population de ce village est exposée pendant toute l'année au risque d'infection par des filaires zoonotiques.*

MOTS CLÉS : *simulies, activité de vol, filaire, infection naturelle, Simuliidae, Thaïlande.*

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INTRODUCTION

Black flies or Simuliidae are known as vectors of certain parasites (Crosskey, 1990). However, no information was available on the vectorial roles of black flies in Asian countries until we reported *Simulium bidentatum* Shiraki, 1935, and six other *Simulium* species to be naturally infected with bovine *Onchocerca* species in relation to the transmission of zoonotic onchocerciasis found in Japan (Takaoka, 1994;

Takaoka *et al.*, 1989, 1992), and *S. uchidai* (Takahasi, 1950) as a vector of an unknown bird filarial species (Fukuda *et al.*, 2005). Recently we experimentally showed six Japanese *Simulium* species as potential vectors of five zoonotic *Onchocerca* species including *O. dewittei japonica* Uni, Bain & Takaoka, 2001 from wild boar, a causative agent of zoonotic onchocerciasis (Fukuda *et al.*, 2008). In Thailand, we found *S. asakoae* Takaoka & Davies, 1995, *S. nodosum* Puri, 1933 and *S. nigrogilvum* Summers, 1911 naturally infected with larvae of different filarial species, though third-stage larvae were recovered only from the latter two *Simulium* species (Fukuda *et al.*, 2003; Takaoka *et al.*, 2003).

This study aimed to clarify the vectorial roles in zoonotic filarial transmission of the two predominant man biting species, *S. asakoae* and *S. nodosum*, throughout the year as well as their seasonal and daily biting activity patterns in northern Thailand.

MATERIALS AND METHODS

Study area

Collections of adult black flies were carried out at the site selected in an open grassland along the stream (6-10 m wide) with moderate current, in the village of Ban Pang Faen (99° 20' E and 18° 50' N: *ca.* 250 m in altitude), Doi Saket district, Chiang Mai Province, northern Thailand, as in our previous study (Takaoka *et al.*, 2003). There were approximately 300 inhabitants and 50 water buffalos, but no cattle in this village.

Collections of adult black flies

Collections were conducted twice a month from 06-00 to 18-00 hours from January 2005 to February 2006, and adult black flies attracted to human bait were captured by a hand net (diameter 30 cm, depth 60 cm). Each collection time consists of 50 minutes for catching and 10 minutes for measuring air temperature, relative humidity and light intensity. Adult black flies caught hourly were preserved in separate vials filled with 80 % ethanol, and brought to the laboratory. The specimens were identified into species using the key provided by Takaoka & Choochote (2004).

The mean total numbers of adult females of each species per day were calculated from the two-day collections of each month to express seasonal biting activities of two predominant species. Daily biting activity patterns were evaluated when the total numbers of adult females per day were over than 50. The times were regarded as peaks when more than 20 % of daily collection was caught.

A year was climatically divided into three seasons on the basis of rainfall and air temperature values, i.e., hot season (from mid February to mid May), rainy season (from mid May to mid October), and dry-cool season (from mid October to mid February), as determined by Thai Meteorological Department. The monthly changes in rainfall, relative humidity and air temperature from January 2005 to February 2006, were measured by the Northern Meteorological Center, situated in Muang district, Chiang Mai Province, at an altitude of 312 m.

Dissections of adult black flies and study of recovered nematodes

After classification to species, individual flies were microscopically dissected and searched for filarial larvae in a drop of 0.5 % Giemsa's stain solution on a slide glass.

Nematodes recovered were classified into non-filarial and filarial worms. The first were only measured. The filarial larvae were classified into first-, second-, and third-stage following Duke (1968) and a morphological study was performed with third-stage larvae fixed in 2 % formalin. Comparison with known species followed Bain & Chabaud (1986) who established diagnostic formulae based on four characters with two states each (A & B). The characters are the following: 1, ratio length tail / body width at anus; 2, body length; 3, ratio esophagus length x 100 / body length; 4, ratio tail length x 100 / body length. The other discriminative characters were the morphology of the tail extremity and nature of vectors (Bain & Chabaud, 1986).

RESULTS

Black fly species collected

Table I shows the total numbers of adult females of ten black fly species collected in this survey. The most abundant species was *S. nodosum*

***Simulium* species**	**No. (%) flies collected**	
S. nodosum	9,487	(57.3)
S. asakoae	6,157	(37.2)
S. nakhonense	783	(4.7)
S. nigrogilvum	83	(0.5)
S. chamlongi	27	(0.2)
S. chumpornense	7	(0.1)
S. fenestratum	4	
S. burtoni	3	
S. doipuiense	1	
S. sheilae	1	
Total	16,553	(100.0)

Table I. – Numbers of black fly females collected at Ban Pang Faen, Chiang Mai Province, in northern Thailand, from January 2005 to February 2006.

(57.3 %) followed by *S. asakoae* (37.2 %). Mean monthly air temperatures, relative humidities and rainfalls recorded by the Northern Meteorological Center were 22.5-29.9°C, 57-88 %, and 0-436.3 mm, respectively. Light intensities measured at the site were 21-117,667 Lux in hot season, 279-118,133 Lux in rainy season, and 0-104,867 Lux in dry-cool season.

SEASONAL BITING ACTIVITY OF *S. NODOSUM* AND *S. ASAKOAE*

The mean monthly numbers of adult females of *S. nodosum* and *S. asakoae* with monthly air temperatures and rainfalls were demonstrated in Fig. 1. Both species showed different patterns in their seasonal biting activities. *S. nodosum* had increased numbers in hot season (86.7 % of the total catch) with a peak in March, and very low numbers in other seasons. On the other hand, *S. asakoae* had relatively high numbers in rainy season (74.5 % of the total catch) with two peaks, first in June and second in September, and relatively low numbers in other seasons.

DAILY BITING ACTIVITY PATTERNS OF *S. NODOSUM* AND *S. ASAKOAE*

Table II and Figure 2 present the daily biting activity patterns of the two species. *S. nodosum* had two patterns: nine of the 15 collections analysed were unimodal with a peak from 17-00 to 18-00, and six were bimodal with one peak from 16-00 to 18-00 and the other from 07-00 to 09-00. The main peaks in bimodal patterns were mostly observed in late afternoon. For *S. asakoae*, 22 of the 25 collections analysed were unimodal with a peak from 06-00 to 10-00, and a few others were bimodal.

NATURAL INFECTIONS WITH FILARIAL LARVAE

Table III shows the results of dissections of females of *S. nodosum* and *S. asakoae*. The monthly average

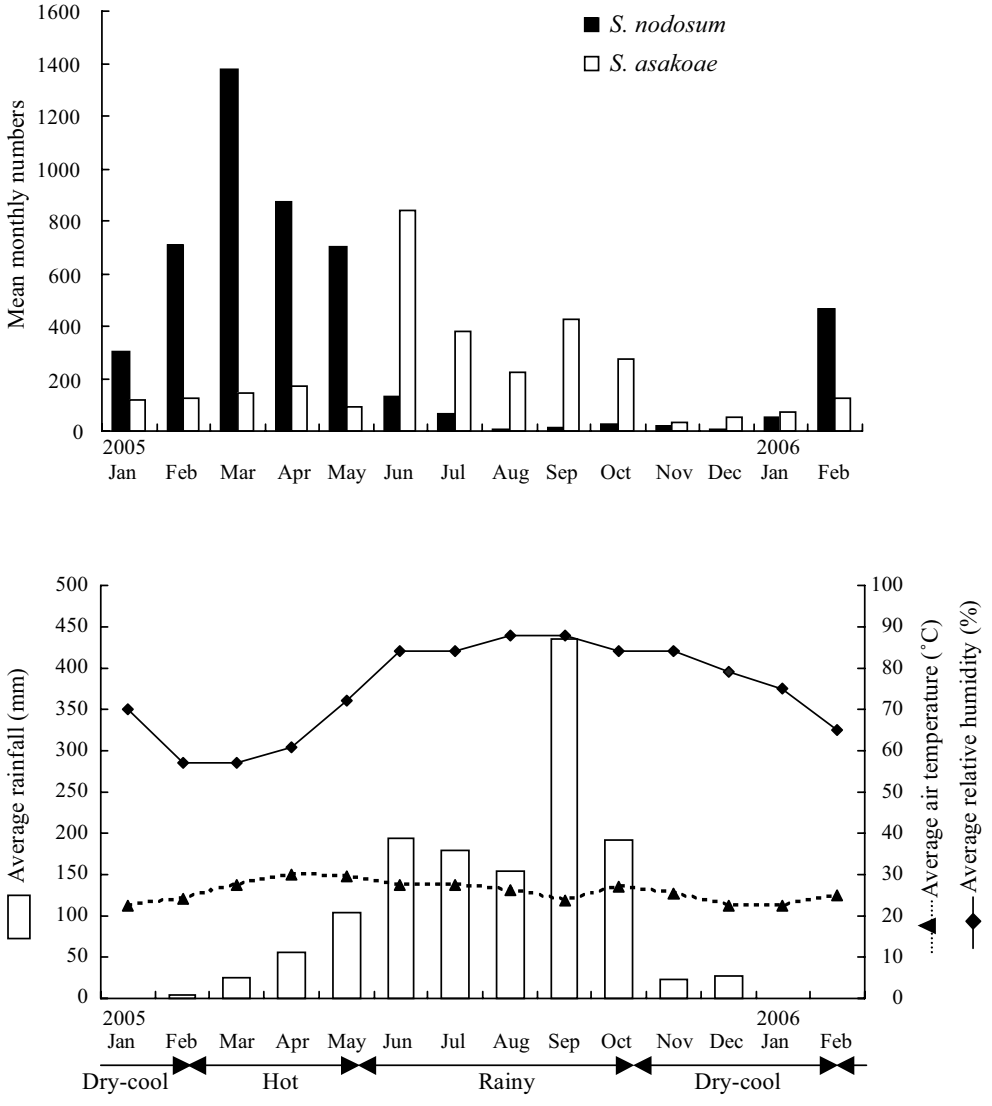

Fig. 1. – Seasonal changes in biting activity of *S. nodosum* and *S. asakoae*, with average air temperature, average relative humidity and average rainfall.

	2005												2006	
	1	2	3	4	5	6	7	8	9	10	11	12	1	2
Species	**Dry-cool**		**Hot**			**Rainy**					**Dry-cool**			
S.nodosum	U 15	B 16 (8)	U 16	B 17 (7)	B 17 (7)	B 17 (7)	–	–	–	–	–	–	–	U 17
	B 16 (8)	B 16 (9)	U 17	B 16 (8)	U 17	B 7 (14)	B 8 (16)	–	–	–	–	–	–	U 17
S. asakoae	B 17 (8)	U 8	U 7	U 7	U 6	U 6	U 6	B 7 (17)	U 7	U 6	–	–	U 9	U 8
	B 8 (17)	U 8	U 7	U 6	U 6	U 6	U 6	U 7	U 6	U 6	–	U 9	U 9	U 8

U, B mean unimodal, bimodal biting activity patterns, respectively.
Numbers mean major peak time (e.g., 16, 16-00 to 17-00 hours).
Numbers in parentheses mean secondary peak time.
Bars indicate "not assessed for biting activity patterns" due to small numbers less than 50 black fly females collected per day.

Table II. - Daily biting activity patterns of two predominant *Simulium* species in two collection days of each month at Ban Pang Faen, Chiang Mai Province, in northern Thailand.

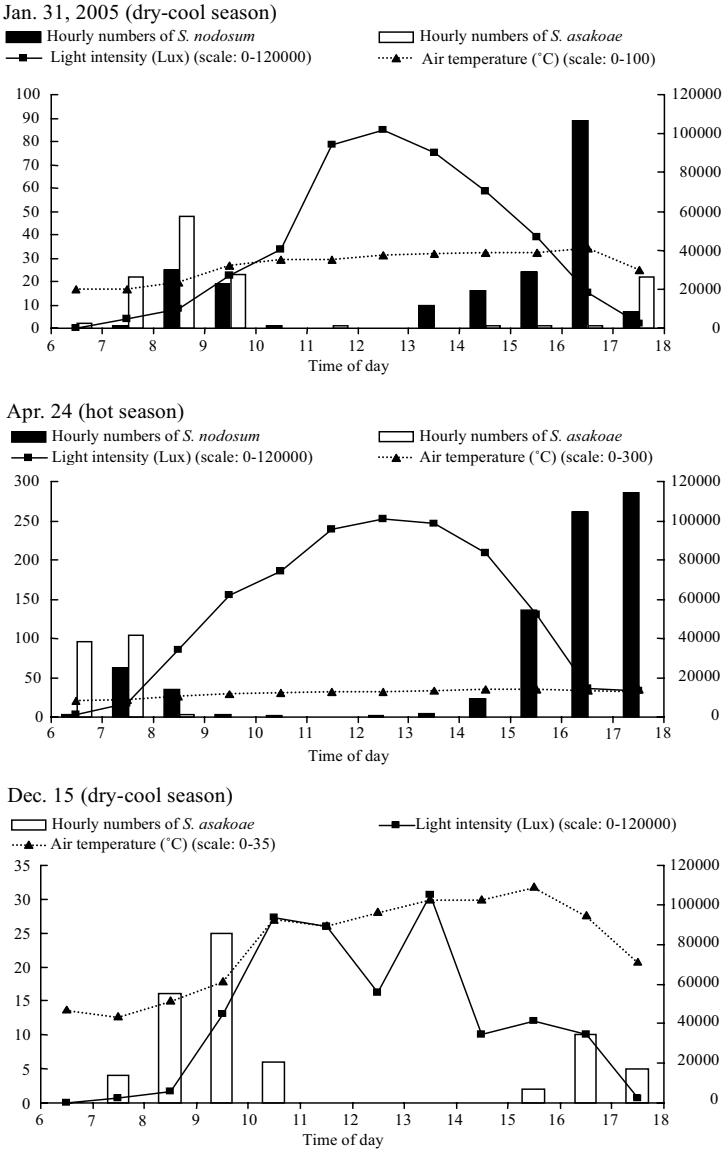

Fig. 2. – Different daily biting activity patterns of *S. nodosum* and *S. asakoae* attracted to a human bait from 06-00 to 18-00 hours at Ban Pang Faen, northern Thailand: bimodal pattern of both species in January (top), bimodal pattern of *S. nodosum* and unimodal pattern of *S. asakoae* in April (middle) and bimodal pattern of *S. asakoae* in December (bottom).

Species	Month	No. collected	No. dissected	No. with filarial			No. with non-filarial nematodes (%)
				L1* (%)	L2* (%)	L3* (%)	
S. nodosum	Jan.2005	603	100	0 (0)	0 (0)	1 (1.0)	37 (37.0)
	Feb.	1,416	100	2 (2.0)	0 (0)	3 (3.0)	8 (8.0)
	Mar.	2,758	201	0 (0)	0 (0)	0 (0)	1 (0.5)
	Apr.	1,750	200	1 (0.5)	1 (0.5)	0 (0)	1 (0.5)
	May	1,402	100	3 (3.0)	0 (0)	0 (0)	0 (0)
	Jun.	255	163	0 (0)	0 (0)	1 (0.6)	1 (0.6)
	Jul.	125	86	1 (1.2)	0 (0)	0 (0)	1 (1.2)
	Aug.	14	14	0 (0)	0 (0)	0 (0)	0 (0)
	Sep.	22	22	0 (0)	0 (0)	0 (0)	0 (0)
	Oct.	47	47	0 (0)	0 (0)	1 (2.1)	3 (6.4)
	Nov.	36	36	0 (0)	0 (0)	0 (0)	26 (72.2)
	Dec.	18	18	0 (0)	0 (0)	0 (0)	5 (27.8)
	Jan.2006	108	108	0 (0)	0 (0)	0 (0)	49 (45.4)
	Feb.	933	100	1 (1.0)	0 (0)	0 (0)	7 (7.0)
S. asakoae	Jan.2005	240	103	0 (0)	0 (0)	2 (1.9)	1 (1.0)
	Feb.	244	104	0 (0)	2 (1.9)	0 (0)	0 (0)
	Mar.	287	100	0 (0)	1 (1.0)	0 (0)	0 (0)
	Apr.	341	100	1 (1.0)	0 (0)	3 (3.0)	0 (0)
	May	190	100	0 (0)	1 (1.0)	0 (0)	0 (0)
	Jun.	1,682	100	0 (0)	0 (0)	0 (0)	0 (0)
	Jul.	765	100	1 (1.0)	1 (1.0)	1 (1.0)	0 (0)
	Aug.	441	100	1 (1.0)	1 (1.0)	0 (0)	0 (0)
	Sep.	856	100	1 (1.0)	0 (0)	0 (0)	0 (0)
	Oct.	555	100	0 (0)	0 (0)	0 (0)	1 (1.0)
	Nov.	60	60	0 (0)	0 (0)	1 (1.7)	0 (0)
	Dec.	109	109	0 (0)	0 (0)	0 (0)	0 (0)
	Jan.2006	138	100	0 (0)	0 (0)	1 (1.0)	0 (0)
	Feb.	249	100	0 (0)	0 (0)	0 (0)	0 (0)

*: L1; first-stage larva. L2; second-stage larva. L3; third-stage larva.

Table III. – Numbers and proportions of females of *S. nodosum* and *S. asakoae* harbouring first-, second- and third-stage filaraial larvae and non-filarial nematodes.

air temperatures of the investigation area were more than 17°C, the minimum air temperature for the larval development of *Onchocerca volvulus* in *S. ochraceum* Walker, 1861 in Guatemala (Takaoka *et al.*, 1982). Natural filaria infections were found almost every month (except December) and their rates were 0.6-5.0 % for *S. nodosum* and 1.0-4.0 % for *S. asakoae*. Among the 15 infected females of *S. nodosum*, six harboured one, two or three third-stage filarial larvae in their head, thorax and/or abdomen. One fly had a second-stage larva in the thorax. Eight other flies harboured one, two or three first-stage filarial larvae in their head or thorax. Four first-stage larvae measured were 200-270 µm long by 9-19 µm wide, and one first-stage larva found in their head was 88 µm long by 9 µm wide. The second-stage larva found in the thorax was 503 µm long by 39 µm wide. Seven other first-stage larvae and six third-stage larvae were broken, then not measured.

For *S. asakoae*, eight of the 18 infected flies harboured one or three third-stage filarial larvae in their head or thorax. Six flies had one or two second-stage larvae in their thorax. Four flies had one first-stage larva in their thorax. The first-stage larvae were 239-308 µm long by 23-26 µm wide. Second-stage larvae were 22-33 µm wide, but their lengths were not measured.

The measurements of the third-stage larvae found in both species are given and compared to larvae found in northern Thailand in the two previous studies (Table IV). Larvae found in *S. nodosum* had similar size to those described by Takaoka *et al.* (2003) in this species from the same area, and tentatively assigned to the genus *Onchocerca*. Larvae found in *S. asakoae* were different and described (registration numbers 283 JW: four larvae, of which two entire; 307 JW: one entire larva). The morphological features were as follows (Fig. 3): Habitus rather stocky (maximum width is 32 µm in the four larvae measured); cephalic part attenuated; body slightly narrower posterior to esophagus. Head round; only cephalic papillae identified. Buccal cavity flattened laterally; conspicuous sclerotized posterior ring of buccal capsule. Excretory cell was round and conspicuous. Esophagus: thin muscular part, thick and long glandular parts; intestine very short, with thick wall and thread-like lumen. Tail long (2 < tail ratio < 3); tail extremity with two dissymmetric ear-shaped lappets, one almost apical and ven-

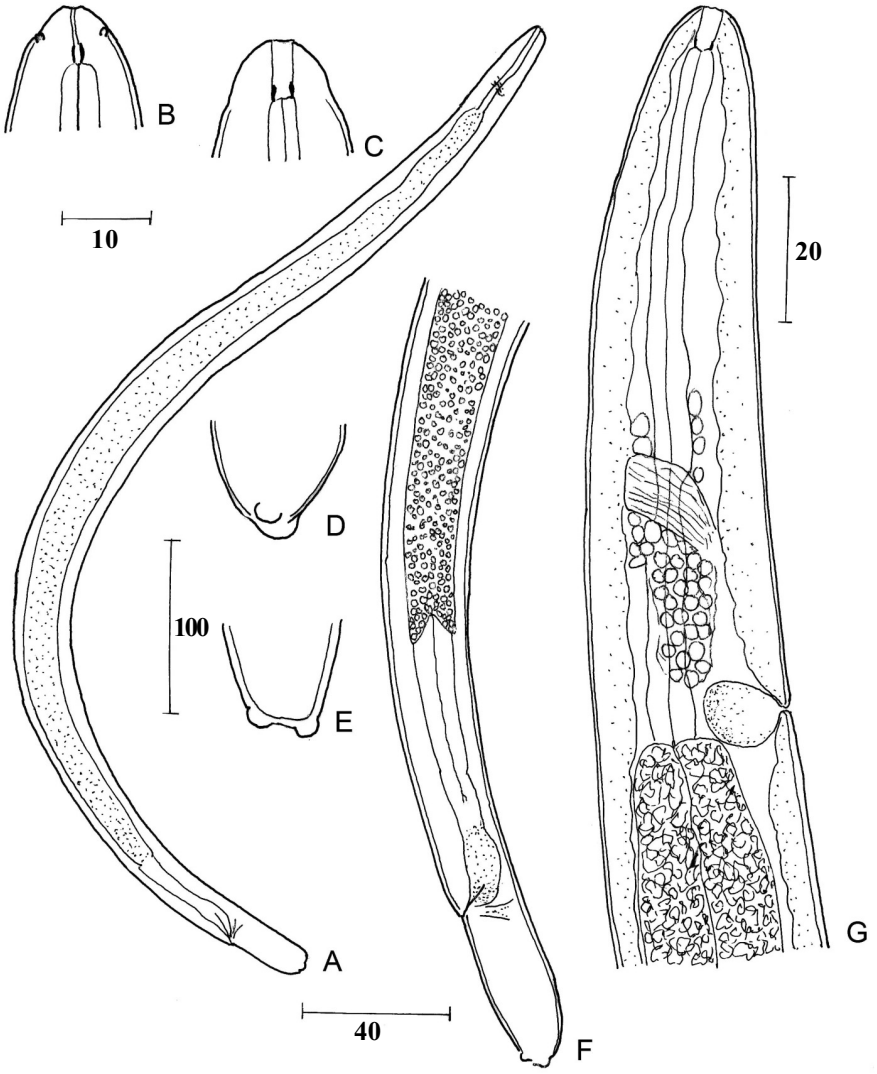

Fig. 3. – Infective larva found in *S. asakoae* at Ban Pang Faen village, in northern Thailand. A: habitus, left lateral view. B and C: anterior extremity, in median and lateral view, respectively. D and E: caudal extremity, right lateral and ventral view, respectively. F: posterior part and esophageal-intestinal junction, left lateral view. G: anterior part, right lateral view. Scales in µm.

Host *Simulium* species	***S. asakoae*[1]**	***S. asakoae*[1]**	***S. asakoae*[1]**	***S. nodosum*[1]**	***S. nodosum*[2]**	***S. nigrogilvum*[3]**
Body length (BL)	640	740	618	997-1,441 (3)	1,315-1,500	800-900
Body width (BW)	32	32	32	23-26 (3)	23-25	25-31
Nerve ring	60	68	60	88 (1)	80-90	ND
Excretory cell	92	92	90	ND	ND	ND
Buccal cavity	6	7	5	ND	4.0-4.5	ND
Esophagus length	510	590	505	ND	690-900	311-336
Intestine	50	40	52	ND	ND	ND
Rectum	28	28	27	ND	ND	ND
Tail length (TL)	50	48	42	43 (2)	45	ND
Tail width at anus (TW)	19	20	19	17-19 (2)	18-25	ND
BWX100/BL	5.0	4.3	5.2	1.8-2.3 (3)	1.6-1.9	2.8-3.9
TLX100/BL	7.8	6.5	6.8	3.0-4.3 (3)	3.0-3.4	ND
Tail ratio (TL/TW)	2.6	2.4	2.2	2.3-2.5 (2)	1.8-2.5	ND
Esophagus/BL (%)	80	80	82	ND	46-62	37-41
Tail shape	cylindrical	cylindrycal	cylindrical	round (3)	round	ND
Caudal end	2 lappets	2 lappets	2 lappets	ND	no or 2 very tiny lappets	ND

[1] Current study: 3/5 larvae with complete measures. [2] Takaoka *et al.* (2003), same study area, on 22 June 2001. [3] Fukuda *et al.* (2003), at Tambol Ban Laung on 16 December 2001.
ND: no data. Numbers of filarial larvae measured are in brackets.

Table IV. – Measurements of infective larvae found in *S. asakoae* and *S. nodosum* collected at Ban Pang Faen, and comparison with larvae previously studied in northern Thailand.

trolateral, one subterminal and lateral. The diagnostic formula of the third-stage larvae found in *S. asakoae* was 1X (= 1A or 1B), 2A, 3B, 4B.

NON-FILARIAL NEMATODES FOUND IN BLACK FLIES

Non-filarial nematodes were found in *S. nodosum* and *S. asakoae* (Table III). At least, two kinds of nematodes were confirmed by the body length, width and morphological features. Natural infection rate of the non-filarial nematodes found in *S. nodosum* was 10.7 % (139/1,295). Most of the infected flies were found in dry-cool season. The highest infection rate was 72.2 % (26/36) in November 2005. While only 0.1 % (2/1,376) of *S. asakoae* were infected with the non-filarial nematodes.

DISCUSSION

Black flies are long known as a serious pest for inhabitants in the village of Ban Pang Faen, northern Thailand. The suspected *Simulium* species has remained undetermined until we reported that *S. nodosum* was the most abundant anthropophilic species in the village when surveyed in June (Takaoka *et al.*, 2003). Dissections of female specimens carried out at the same time further disclosed that *S. nodosum* was naturally infected with larvae of a zoonotic filaria provisionally assigned to *Onchocerca* sp. (Takaoka *et al.*, 2003). Subsequently, further investigation was stimulated to clarify the vectorial roles of *S. nodosum* in zoonotic filaria transmission throughout the year as well as its seasonal and daily biting activity patterns in the village. In the course of the present survey, it was soon revealed that besides *S. nodosum*, *S. asakoae* was also an important man-biting species in the village although this species was known to be ornithophilic (Unpublished data).

Furthermore, the seasonal patterns in biting activity of *S. nodosum* and *S. asakoae* were quite different from each other, as shown in Fig. 1. The difference is presumed to be closely associated with seasonal variations in the availability of streams suitable for the breeding of the two species, which choose differently their aquatic sites (Takaoka, 1981). The main channels of rivers are mostly favored by *S. nodosum* but not by *S. asakoae*, while small streams are preferred by *S. asakoae* but not by *S. nodosum* (Unpublished data). In rainy season, frequent heavy rainfalls and subsequent increased water discharges may cause the rivers unstable for larval breeding of *S. nodosum*, although they may provide numerous small tributary streams which are suitable for the larval breeding of *S. asakoae*. On the other hand, in hot season when no rainfalls, the main channels of rivers will become stable for the breeding of *S. nodosum* but most of small streams will dry out, decreasing the breeding sites of *S. asakoae*. It is however difficult to explain why the adult populations of both species were so low in dry-cool season. The seasonal patterns in the biting activity of adult females of *S. asakoae* attracted to human bait surveyed at Doi Inthanon National Park, northern Thailand, were reported to be different to some extent by altitude (Choochote *et al.*, 2005). The seasonal biting activity patterns obtained by using a human bait may be also influenced by other factors such as availabilities of host animals as a bloodmeal source near the collection site if *Simulium* species concerned are zoophilic as well as anthoropophilic. The daily biting activity patterns of *S. nodosum* and *S. asakoae* also differed (Table II). The fact that *S. nodosum* had a major peak in the late afternoon suggests that adult females of this species are more tolerant to high air temperatures. On the other hand, *S. asakoae*, which usually had a major peak in the early morning, is probably less tolerant to high air temperature and also to high light intensities. The similar unimodal daily biting activity pattern with a morning peak was already reported for *S. asakoae* at low to medium altitudes of Doi Inthanon National Park (Choochote *et al.*, 2005). More data, such as physiological ages of the flies, are necessary to analyse the daily biting activity patterns of each of both species. Third-stage filarial larvae were found for the first time from *S. asakoae*. They were clearly different in body size from those previously reported from *S. nodosum* in that place (Takaoka *et al.*, 2003) and from *S. nigrogilvum* in Tambol Ban Laung (Fukuda *et al.*, 2003), as shown in Table IV. Filaria species with infective larvae presenting the same diagnostic formula (1X, 2A, 3B, 4B) and found in simuliids are few: the South American human parasite *Mansonella (M.) ozzardi* (Manson, 1897), a *Dirofilaria* species from a bear, a *Splendidofilaria* species from birds. The present species is opposed to these species mainly by the body thick (32 µm) but rather short, the large glandular esophagus and very short intestine. In fact, this larva is different from all the previously described third-stage larvae analyzed in Bain & Chabaud (1986) as well as those later published: those from bird parasites of the genera *Pelecitus* Railliet & Henry, 1910 and *Eulimdana* Founikoff, 1934, both transmitted by lice (Bartlett & Anderson, 1987b; Bartlett, 1993), and those of the *Onchocerca* species experimentally obtained (Fukuda *et al.*, 2008). It is not possible to identify the filarial larvae from *S. asakoae* due to the limited data on the infective larvae (about one third of the onchocercid genera) and the lack of knowledge on the local filarial parasites. However, since the study area is rural, it is expected that the domestic mammals, water buffalo, cattle, dogs, horses, have no other onchocercid

species than those which belong to the common genera *Setaria* Viborg, 1795, *Onchocerca* Diesing, 1841 and *Dirofilaria* Railliet & Henry, 1910: their third-stage larvae are different from the present one. We suggest that birds might be the reservoir of the new particular filaria species as *S. asakoae* feeds birds: avian filariae are diverse (Bartlett & Anderson, 1987a) and their third-stage larvae often half known (9/16 genera). The majority of bird onchocercids belong to the Splendidofilariinae Chabaud & Choquet, 1953 and the Lemdaninae Lopez-Neyra 1956 (Anderson & Bain, 1976). Some genera, with unknown infective larvae, have a large esophagus, *Paronchocerca* Peters, 1936 and *Aproctella* Cram, 1931 in the first subfamily, *Aproctiana* Skrjabin, 1934 and *Lemdana* Seurat, 1917 in the second one (Anderson & Bain, 1976). The last genus is well represented in the Oriental Region, parasitic in Galliformes and other birds (Bartlett & Anderson, 1987a). These comments do not exclude that the filaria species found in *S. asakoae* might belong to a new genus.

The third-stage larvae found from *S. nodosum* in this study were thought to belong to the same filaria species previously found from the same black fly species in the same village and provisionally assigned to the genus *Onchocerca* (Takaoka *et al.*, 2003), a parasite of ungulates; this fits with the mammophilic habits of *S. nodosum*. Final identification at genus and species level remains undetermined due to the lack of the adult filariae.

It is concluded that in Ban Pang Faen, northern Thailand, *S. nodosum* is a major man-biter in hot season but replaced by *S. asakoae* in rainy season, and that both species differ from each other with regard to their seasonal and daily biting activity patterns and filaria species they transmit. Taking natural filarial infections of the two black fly species together, it is also considered that people in the village are likely to be exposed to the risk of infection with zoonotic filariae throughout the year.

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REFERENCES

ANDERSON R. C. & BAIN O. Key to the genera of the order Spirurida. Part 3. Diplotriaenoidea, Aproctoidea and Filarioidea, *in*: Anderson R.C., Chabaud A.G. & Willmott S. (Eds) CIH keys to the nematode parasites of vertebrates. Farnham Royal: CAB, 1976, No 3, pp. 59-116.

BAIN O. & CHABAUD A.G. Atlas des larves infestantes de Filaires. *Tropical Medicine and Parasitology*, 1986, *37*, 301-340.

BARTLETT C.M. Lice (*Amblycerra* and *Ischnocerca*) as vectors of *Eulimdana* spp. (Nematoda: Filarioidea) in charadriform birds and the necessity of short reproductive periods in adult worms. *Journal of Parasitology*, 1993 *79*, 85-91.

BARTLETT C.M. & ANDERSON R.C. *Lemdana wernaarti* n. sp. and other filarioid nematodes from *Bubo virginianus* and *Asio otus* (Strigiformse) in Ontario, Canada, with a revision of *Lemdana* and a key to avian filarioid genera. *Canadian Journal of Zoology*, 1987a, *65*, 1100-1109.

BARTLETT C.M. & ANDERSON R.C. *Pelecitus fulicaeatrae* (Nematoda: Filarioidea) of coots (Gruiformes) and grebes (Podicipiformes): skin-inhabiting microfilariae and development in Mallophaga. *Canadian Journal of Zoology*, 1987b, *65*, 2803-2812.

CHOOCHOTE W., TAKAOKA H., FUKUDA M., OTSUKA Y., AOKI C. & ESHIMA N. Seasonal abundance and daily flying activity of black flies (Diptera: Simuliidae) attracted to human baits in Doi Inthanon National Park, northern Thailand. *Medical Entomology and Zoology*, 2005, *4*, 335-348.

CROSSKEY R.W. The natural history of Blackflies. The Natural History Museum, London, 1990.

DUKE B.O.L. Studies on factors influencing the transmission of onchocerciasis. V: The stages of *Onchocerca volvulus* in wild forest *Simulium damnosum*, the fate of the parasites in the fly and the age-distribution of the biting population. *Annals of Tropical Medicine and Parasitology*, 1968, *62*, 107-116.

FUKUDA M., BAIN O., AOKI C, OTSUKA Y. & TAKAOKA H. Natural infections of *Simulium* (*Nevermannia*) *uchidai* (Diptera: Simuliidae) with infective filarial larvae, probably from a bird, in Oita, Japan. *Medical Entomology and Zoo*logy, 2005, *56*, 93-98.

FUKUDA M., CHOOCHOTE W., BAIN O., AOKI C. & TAKAOKA H. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Japanese Journal of Tropical Medicine and Hygiene*, 2003, *31*, 99-102.

FUKUDA M., TAKAOKA H., UNI S. & BAIN O. Infective larvae of five *Onchocerca* species from experimentally infected *Simulium* species in an area of zoonotic onchocerciasis in Japan. *Parasite*, 2008, *15* (in press).

TAKAOKA H. Seasonal occurrence of *Simulium ochraceum*, the principal vector of *Onchocerca volvulus* in the southeastern endemic area of Guatemala. *American Journal of Tropical Medicine and Hygiene*, 1981, *30*, 1121-1132.

TAKAOKA H. Natural vectors of three bovine *Onchocerca* species (Nematoda: Onchocercidae) and their seasonal transmission by three blackfly species (Diptera: Simuliidae) in central Kyushu, Japan. *Journal of Medical Entomology*, 1994, *31*, 404-416.

TAKAOKA H., AOKI C. & HAYAKAWA H. Natural infections of blackflies with larvae of zoonotic *Onchocerca* spp. in nor-

theast Japan. *Japanese Journal of Tropical Medicine and Hygiene*, 1992, *20*, 1-9.

TAKAOKA H., BABA M. & BAIN O. Natural infections of *Simulium bidentatum* (Diptera: Simuliidae) with larvae of *Onchocerca* spp. in relation to a human zoonotic onchocerciasis in Oita, Japan. *Japanese Journal of Tropical Medicine and Hygiene*, 1989, *17*, 279-284.

TAKAOKA H. & CHOOCHOTE W. A list of and keys to black flies (Diptera: Simuliidae) in Thailand. *Tropical Medicine and Health*, 2004, *32*, 189-197.

TAKAOKA H., CHOOCHOTE W., AOKI C., FUKUDA M. & BAIN O. Black flies (Diptera: Simuliidae) attracted to human and water buffalos and natural infections with filarial larvae, probably *Onchocerca* sp., in northern Thailand. *Parasite*, 2003, *10*, 3-8.

TAKAOKA H., OCHOA J.O., JUAREZ E.L. & HANSEN K.M. Effects of temperature on development of *Onchocerca volvulus* in *Simulium ochraceum*, and longevity of the simuliid vector. *Journal of Parasitology*, 1982, *68*, 478-483.

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