BLACK FLIES (DIPTERA: SIMULIIDAE) ATTRACTED TO HUMANS AND WATER BUFFALOS AND NATURAL INFECTIONS WITH FILARIAL LARVAE, PROBABLY *ONCHOCERCA* SP., IN NORTHERN THAILAND

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Summary:

Several *Simulium* species were investigated as to their biting habits and natural infections with filarial larvae at Ban Pan Fan, Chiang Mai Province, in northern Thailand. Female adults flies landing on or flying around a human and a water buffalo were collected during the daytime from 06.00 to 19.00 hours on 22 June 2001. As a result, 217 *S. nodosum*, 86 *S. asakoe* and two *S. nigrogilvum* were obtained from a human attractant, and 416 *S. nodosum*, 25 *S. nakhonense*, 16 *S. asakoe*, four *S. fenestratum* and two *S. nigrogilvum*, from a water buffalo. The blood-feeding was confirmed only for *S. nodosum* and *S. nigrogilvum* on humans, and for *S. nodosum* and *S. nakhonense* on water buffalos. Dissections of these simuliids showed that *S. nodosum* was naturally infected with developing filarial larvae. Two types of microfilariae were distinguished but only one type of infective larvae. These larvae resembled *Onchocerca suzukii*, a parasite from a wild Japanese bovid, suggesting that an unknown *Onchocerca* species from ruminants was transmitted in Thailand. Infection rates with all stages of larvae and third-stage larvae were 2.3 % (14/608) and 1.0 % (6/608), respectively. This is the first report of natural infections of black flies with *Onchocerca* larvae in Southeast Asia, and the involved black fly species is shown to be not only anthropophilic but also zoophilic in this region.

KEY WORDS : black fly, filaria, natural infection, *Onchocerca*, Simuliidae, Thailand.

Résumé : SIMULIES ATTIRÉES PAR L'HOMME ET LE BUFFLE DOMESTIQUE EN THAILANDE SEPTENTRIONALE ET INFESTATIONS NATURELLES PAR DES LARVES DE FILAIRES, PROBABLEMENT D'*ONCHOCERCA* SP.

Identification et préférence trophique des espèces de simuliées capturées à Ban Pan Fan (Province de Chiang Mai) et étude de leur infestation naturelle par des larves de filaires. Les femelles des simuliées volant autour ou se posant sur l'homme ou sur un buffle domestique ont été capturées le 22 juin 2001 de 6 h à 19 h. 217 *S. nodosum*, 86 *S. asakoe* et deux *S. nigrogilvum* ont été obtenues avec l'appât humain ; 416 *S. nodosum*, 25 *S. nakhonense*, 16 *S. asakoe*, four *S. fenestratum* et deux *S. nigrogilvum* avec l'appât buffle. Quelques simuliées se sont gorgées : *S. nigrogilvum* sur l'homme, *S. nakhonense* sur le buffle et *S. nodosum* sur les deux. Chez *S. nodosum*, deux types de microfilaries (2,3 % = 14/608) et un type de larve infestante (1 % = 6/608) ont été trouvés. Les larves infestantes ressemblent à celles d'*Onchocerca suzukii*, qui est parasite d'un Bovidae sauvage du Japon. Cela suggère l'existence d'une espèce encore inconnue en Asie du Sud-est, et on montre que la similie incriminée est non seulement anthropophile, mais aussi zoophile dans cette région.

MOTS CLÉS : similie, filaire, infection naturelle, *Onchocerca*, Thaïlande.

INTRODUCTION

Simuliidae or black flies are commonly encountered in many areas around the world where running waters as an aquatic habitat for their larval and pupal stages are available. Female adults of some simuliid species have been well known to be a pest of humans and animals and also vectors of some parasites and pathogens, like *Onchocerca* spp. in

humans and cattle and *Leucocytozoon* spp. in birds (Crosskey, 1990).

In the Oriental Region, several man-biting simuliid species have been known, e.g., *Simulium indicum* Becher, 1885 widely distributed at the southern foot of the Himalayas, from Pakistan in the west, through India and Nepal, to Myanmar and southern China in the east (Lewis, 1974; Datta, 1992), *S. asisbi* Datta, 1988, *S. himalayense* Puri, 1932, *S. nodosum* Puri, 1933, *S. tenuistylum* Datta, 1973 in India (Datta, 1992), and *S. japonicum* Matsuura, 1931 in the Ryukyu Islands, Japan (Takaoka, 1977); several other species have been reported as a pest of animals in Java (Friederichs, 1925) and in India (Datta, 1992; Datta & Dasgupta, 1975). In Thailand, no such information on feeding habits of female black flies was available so far, though the simuliid fauna of this country has been extensively explored recently, bringing the total number of black-fly species up to 44 (Kuvangkadilok & Takaoka, 2000; Takaoka, 2001; Takaoka & Choochote, 2002; Takaoka & Kuvangkadilok, 1999).

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In northern Thailand, we collected and identified adult females of simuliid species on a human and a water buffalo as an attractant and, by dissecting them, searched for their natural filarial infection.

MATERIALS AND METHODS

STUDY AREA

Collections of adult black flies were conducted at an open grassland along the stream (6-10 m wide) with moderate current, running in the village of Ban Pan Fan (18° 50' E and 99° 20' N: ca. 250 m in altitude), Doi Saket District, Chiang Mai Province, northern Thailand. At least five simuliid species were confirmed to breed in this stream; i.e., *S. nodosum*, *S. nakbonense* Takaoka & Suzuki, 1984, *S. asakoe* Takaoka & Davies, 1995, *S. fenestratum* Edwards, 1934 and *S. parahiayangum* Takaoka & Sigit, 1992 in this order of dominance. There were ca. 300 inhabitants and ca. 50 water buffalos but no cattle in this village.

COLLECTIONS OF ADULT BLACK FLIES

Two collections were undertaken: the first collection was a preliminary one, aimed to know what black-fly species are attracted to a human and a water buffalo, and the second and main one, to observe whether there are any differences among black-fly species in the host preference, and in the time for their host-seeking flight activity within a daytime.

The first collection was carried out on 22 June 2001, for two hours from 10.00 to 12.00 hours using a water buffalo tethered to the tree as an attractant, and for six hours from 12.00 to 18.00 hours using a human attractant (WC, one of the authors) with his legs below the knees exposed. Female black flies landing (or feeding) on or flying around each attractant were caught by an insect net, and killed and held in a vial with 70 % ethanol solution. In the second collection, a human (HT, one of the authors) and a water buffalo, both of which were located ca. 15 m apart from each other, were used at the same time as an attractant. Collections were made for 45 minutes per every hour from 06.00 to 19.00 hours on 22 June 2001, and female black flies hourly caught on each attractant were killed and preserved, as mentioned above. Air temperature was measured and recorded every hour from 06.00 to 19.00 hours.

DISSECTIONS OF ADULT BLACK FLIES FOR FILARIAL LARVAE

After classification to species using the descriptions of Puri (1933) and Takaoka & Suzuki (1984), most of flies were microscopically dissected and searched for fila-

rial larvae in a drop of Giemsa's staining solution on a slide glass. The filarial larvae found were preserved in 2 % formalin solution for morphometric observations. Generic diagnosis of third-stage filarial larvae followed Bain & Chabaud (1986).

MICROFILARIAL INTAKE

Among the female flies collected from a water buffalo, those that were fully engorged were separated, and the bloodmeal (in the midgut) of each fly was dissected into pieces to search for microfilariae ingested. The method of staining and dissection of blood-fed flies followed Nakamura (1964).

RESULTS

BLACK FLY SPECIES COLLECTED

In the first collection, two blood-fed and 366 unfed females of *S. nodosum* and four unfed females of *S. asakoe* were caught from a human attractant, while two blood-fed and 22 unfed females of *S. nodosum* were collected from a water buffalo.

In the second collection, a total of 305 females of three black-fly species were collected on a human attractant, and *S. nodosum* was in the majority (71.1 %), followed by *S. asakoe* (28.2 %) and *S. nigrogilvum* Summers, 1911 (0.7 %), as shown in Table I. Several flies were allowed to achieve engorgement of blood on a human attractant (HT), and feeding on a human blood was confirmed by this direct observation for *S. nodosum* and *S. nigrogilvum* but not for *S. asakoe*. On the other hand, a total of 463 female flies belonging to five black-fly species were collected from a water buffalo. *S. nodosum* was also the most abundant species occupying 89.8 % of the total catch and all the other species were few, consisting of 0.4-5.4 % (Table I). Freshly-ingested bloodmeal in the fly's midgut was recognized in 20.2 % (84/416) of *S. nodosum* and in 8.0 % (2/25) of *S. nakbonense*, while no bloodmeal was detected in the other three species.

DIURNAL BLOOD-SOURCE SEEKING ACTIVITY PATTERN AND HOST PREFERENCE

The weather was fine, and the air temperature fluctuated between 19.0°C and 28.5°C when the second collection was performed. The blood-source seeking activity of *S. nodosum* under these conditions began early in the morning and persisted during the daytime until the late afternoon, though the number of flies captured per 45 minutes varied from 1 to 63; while that of *S. asakoe* appeared to be restricted to the early morning and the late afternoon, though one female was collected late in the morning (between 11.00 and 11.45 hours) on a

Hour	Human <i>Simulium</i> spp. #1			Water buffalo <i>Simulium</i> spp. #1					Air
	A	B	C	A	B	C	D	E	Temp. °C
06-07	1	51	1	28	5	1	8	2	19.0
07-08	13	26	1	52	2	1	9	2	20.0
08-09	25	1	0	39	0	0	4	0	23.0
09-10	11	0	0	13	0	0	2	0	25.6
10-11	1	0	0	2	0	0	2	0	27.0
11-12	3	0	0	31	1	0	0	0	26.8
12-13	3	0	0	11	0	0	0	0	25.8
13-14	11	0	0	61	0	0	0	0	27.8
14-15	29	0	0	53	0	0	0	0	27.0
15-16	17	0	0	31	0	0	0	0	26.0
16-17	4	0	0	22	0	0	0	0	28.5
17-18	36	0	0	45	0	0	0	0	28.2
18-19	63	8	0	28	8	0	0	0	26.8
Total	217	86	2	416	16	2	25	4	
% catch	71.1	28.2	0.7	89.8	3.5	0.4	5.4	0.9	

#1. A, *Simulium nodosum*; B, *S. asakoe*; C, *S. nigrogilvum*; D, *S. nakbonense*; E, *S. fenestratum*.

Table I. – Numbers of female adult black flies collected on a human and a water buffalo, at Ban Pan Fan, Chiang Mai Province, northern Thailand, on June 22, 2001.

Attractants	No. flies dissected	No. flies positive (%)	No. flies with each larval stage ^{#1}				
			Mf	L1	L2	L3	(%)
		<i>S. nodosum</i>					
Human	377	9 (2.4)	0	3	3 ^{#2}	4 ^{#2}	(1.1)
Water buffalo	231	5 (2.2)	1	2	0	2	(0.9)
		<i>S. asakoe</i>					
Human	84	0 (0.0)	—	—	—	—	(—)
		<i>S. nakbonense</i>					
Water buffalo	25	0 (0.0)	—	—	—	—	(—)

^{#1} Mf, microfilaria; L1, first-stage larva; L2, second-stage larva; L3, third-stage larva.

^{#2} One fly had an L3 and an L2 larvae.

Table II. – Results of dissections of females of three black-fly species collected from a human and/or a water buffalo, at Ban Pan Fan, Chiang Mai Province, northern Thailand, for natural filarial infections.

water buffalo. Three other species appeared to be attracted only in the early and/or mid morning.

The total numbers of female flies collected on a human and a water buffalo were compared to each other for *S. nodosum* and *S. asakoe*, and the results show that the former species is 1.9 times more zoophilic and the latter is 5.4 times more anthropophilic.

NATURAL INFECTIONS WITH INFECTIVE AND DEVELOPING FILARIAL LARVAE

As shown in Table II, natural infections of *S. nodosum* with filarial larvae were found in 2.4 % (9/377) of female flies captured on a human, and in 2.2 % (5/231) collected on a water buffalo (combined infection rates with all stages of larvae and third-stage larvae were 2.3 % and 1.0 %, respectively), while no infection was found in all the 84 *S. asakoe* and 25 *S. nakbonense* dissected.

Among the 14 infected females of *S. nodosum*, five flies harboured one or three third-stage filarial larvae in either their head or thorax or abdomen. One fly had one second-stage larva in the thorax as well as one third-stage larva in the head. Eight other infected flies harboured in their thorax one unsheathed microfilaria or one first-stage larva or one second-stage larva.

All the third-stage larvae found have similar size (Table III) and morphology (Photo 1, Figs. A-G), which are described as follows: large infective larva. Anterior and posterior region of same width, or anterior region from the nerve ring to head slightly attenuated. Head conical; four labial and four salient cephalic papillae; amphids clearly defined with their depressed openings. Esophagus divided, with thick glandular part; it has not been possible to localize the esophageal-intestinal junction on any of the specimens, and the length of the esophagus appeared to be grossly measurable

Photos 1-3. – Filarial larvae found in females of *Simulium nodosum*. 1, third-stage larva found in the fly's head (Scale bar, 100 μ m); 2, microfilaria found in the fly's thorax (Scale bar, 100 μ m); 3, microfilaria found in the blood meal ingested in the fly's midgut (Scale bar, 20 μ m).

only at a low magnification: the intestine begins when the granular aspect of the glandular esophagus disappears; an error of 100 μ m in the measurements was estimated by comparing several drawings of each larva. Tail cylindrical, not attenuated, with rounded extremity; a tiny axial terminal round point, rarely well salient and teat-like; lateral lappets rarely identified, and inconspicuous when present.

Fig. 1. – Infective larva. A: Anterior part, right lateral view. B and C: Head, median and lateral view, respectively; E: Posterior part, left lateral view; F: Tail, ventral view; G and H: Caudal extremity of two other specimens, ventral view. Scales: A, 50 μ m; B & C, 10 μ m; others, 20 μ m.

Two first-stage larvae (measured) were 164-190 μ m long by 18-28 μ m wide. The microfilaria found in the thorax (Photo 2) was 390 μ m long by 9 μ m wide; other

Measurements	<i>Onchocerca</i> sp.				<i>O. suzukii</i>
4 larvae from 3 <i>S. nodosum</i>	1	2a	2b	3	Extreme values
Length	1,500	1,460	1,420	1,315	1,075-1,380
Width	25	25	23	25	24-26
Nerve ring-head	80	85	82	90	70-80
Buccal cavity	4	4.5	4	4	5.5
Esophagus	690-800	800-900	750-820	?	500-650
Muscular esophagus	155	125	140	150	115
Tail L/W	45/18	45/20	45/25	45/25	38-45/20
Axial caudal point	round	round	absent	round	round
Lateral lappets	no	no	no	2 very tiny	2 very tiny

Table III - Measurements of infective larvae of *Onchocerca* sp. from *S. nodosum* in Thailand and *O. suzukii* from *Simulium* spp. in Japan.

larval specimens including second-stage larvae were broken, then their body length not measured.

MICROFILARIAL INTAKE

Microfilarial intake was found in two (2.4 %) of 84 blood-fed females of *S. nodosum* collected from a water buffalo. Two flies had, each, one unsheathed microfilaria in the bloodmeal (ingested in their midgut). One microfilaria (Photo 3) measured was 290 µm long by 7 µm wide; the other was cut off, then not measured. Neither of the two blood-fed females of *S. nakbonense* had a microfilaria.

DISCUSSION

In the present study, we found that in Ban Pan Fan Village, Chiang Mai Province, northern Thailand, females of three simuliid species, *S. nodosum*, *S. asakoe* and *S. nigrogilvum*, were attracted to both humans and water buffalos, and two more species, *S. nakbonense* and *S. fenestratum*, only to the latter. Actual blood-feeding was confirmed only for *S. nodosum* and *S. nigrogilvum* on humans, and for *S. nodosum* and *S. nakbonense* on water buffalos. The blood-feeding habits of all these three black-fly species were recorded for the first time except *S. nodosum*, which was previously reported to feed on humans in India (Datta, 1992).

In Chiang Mai Province, a considerable number of *S. nodosum* were attracted to feed on both humans and buffalos, and 2.3 % of them were carrying filarial larvae.

Identifying larval stages of filariae is difficult, particularly when the local parasitic fauna is not well known however some features were assessed.

It is evident that, at least, two filarial species were involved since two types of microfilariae were distinguished. The one found in the ingested blood from the water buffalo could be an *Onchocerca* species. *O. cebei* Galliard, 1937 (= *O. sweetae* Spratt & Moorhouse, 1971, in Bain & Chabaud, 1986), has been identified from water buffalos in Thailand (Bain *et al.*, 1980), but its microfilaria is shorter (215-245 µm). *O. gibsoni* Cleland & Johnston, 1910 is very likely present in Thailand as in other parts of the Oriental Region; its microfilaria has similar length (264-295 µm long) but it seems thinner (3.6-5 µm wide) than the present specimen. The microfilaria found in the thorax was derived from a previous meal taken from an unknown mammalian host and it is not possible to identify it, nor the developing filarial larvae.

It is also clearly shown that one filarial species could complete its larval development in *S. nodosum* but it is not known if these larvae correspond to any microfilarial

type. The infective larvae resemble *Onchocerca* type I, one of the three bovine *Onchocerca* species transmitted by *Simulium* spp. in Kyushu, Japan (Takaoka & Bain, 1990; Takaoka, 1994), which was later identified by Uni *et al.* (1998) to *O. suzukii* Yagi, Bain & Shoho, 1994. This parasite of the Japanese caprine bovid, *Capricornis crispus*, is also transfuge in cattle in Kyushu (Uni *et al.*, 1998). *O. suzukii* infective larvae and the present specimens have large body (> 1 mm long) and a cylindrical long thick tail (tail ratio 1.7-2.2) with round axial terminal point. However the Thai specimens are distinct because they are slightly longer (1,315-1,500 compared to 1,075-1,380 µm). The filarial species which is transmitted by *S. nodosum* in northern Thailand is thus very likely a new *Onchocerca* species which is close to the parasite of the Japanese serow, and still to be discovered in local ruminants.

Zoonotic onchocerciasis appears to be very rare and has so far attracted little attention in Asian countries, but three human cases were recently reported from Kyushu, Japan (Hashimoto *et al.*, 1990; Takaoka *et al.*, 1996; Takaoka *et al.*, 2001): one case probably due to *O. gutturosa* Neumann, 1910, a parasite of cattle, and two other cases due to *O. dewittei japonica* Uni, Bain & Takaoka, 2001, a parasite of wild boar. Similar zoonotic onchocerciasis may occur through the bites of *S. nodosum* in some rural areas of northern Thailand, since this is one of the common black fly species in lowland plains and hilly areas in northern Thailand (Takaoka & Saito, 1996) and shows not only a zoophily but also an anthropophily, as observed in this study.

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