Occurrence of *Leptotrombidium scutellare* larvae in Kagoshima and Nagasaki Prefectures

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Abstract: The occurrence of larval trombiculid mites in different vegetation types was surveyed in Kagoshima and Nagasaki Prefectures. In Kagoshima Prefecture, 10 species were collected at three sites. *Leptotrombidium scutellare* was dominant, followed by *L. pallidum* and *L. fuji*. *L. scutellare* and *L. pallidum* were collected in three vegetation types. The biggest numbers of both species were recorded at the natural woods of two sites and at the grassland of one site. In Nagasaki Prefecture, four species were collected at three sites, and *L. scutellare* was also dominant. *L. scutellare* was collected in two vegetation types. The greater number of *L. scutellare* was recorded at the grassland of two sites and at the natural woods of one site. The distribution of *L. scutellare* larvae was also surveyed along roads passing through hilly and mountainous areas of both prefectures. In Kagoshima Prefecture, the collection of larval trombiculid mites with a black cloth (Suzuki's method) was carried out at 52 sites along five routes, and *L. scutellare* was found in 41 sites (78.8%). In Nagasaki Prefecture, the collection of larval trombiculid mites was carried out at 43 sites along five routes, and *L. scutellare* was found in 4 sites (9.3%). The wide occurrence and high density of vector mites seems to relate to the number of patients in Kagoshima Prefecture.

INTRODUCTION

A remarkable number of tsutsugamushi disease patients have annually been reported in Kagoshima Prefecture since 1980, and the annual incidence of the disease is currently about a hundred. Tsutsugamushi disease patients were reported throughout the mainland Kagoshima Prefecture. The principal vector species that actually transmit *Orientia tsutsugamushi* to humans in Japan are considered to be

the three species, *Leptotrombidium akamushi*, *L. pallidum* and *L. scutellare* (Kawamura et al., 1995). The fauna and seasonal occurrence of trombiculid mites were examined in the high endemic areas of tsutsugamushi disease, and *L. scutellare* and *L. pallidum* were highly suspected as the vector of tsutsugamushi disease in Kagoshima Prefecture (Noda et al., 1996). Recently, unfed larva of *L. scutellare* collected from an endemic area tested positive for *O. tsutsugamushi* (Takahashi et al., 2002).

It is important to explain the reason for the occurrence of the remarkable number of tsutsugamushi disease patients and the wide distribution of patients in Kagoshima Prefecture. Ecological information regarding vector trombiculid mites is insufficient in Kagoshima Prefecture. Especially, data on suitable habitats and precise distributions of vector trombiculid mites are necessary. It was pointed out that larvae of *L. scutellare* and *L. pallidum* seemed to be able to adapt to various habitats, from dry and sunny to wet and shaded (Noda et al., 1996). In the present study, the occurrence of larval trombiculid mites in different vegetation types was examined in Kagoshima and Nagasaki Prefectures to confirm the previous observation. It is very hard to examine vector trombiculid mites over a wide area by separating them from soil samples and animals. A useful and simple sampling method for *L. scutellare* larvae using a black cloth was proposed by Suzuki (1986). Small clusters and solitary larvae were collected frequently with a black cloth from the sites where they were not seen with the naked eye (Uchikawa et al., 1993, designated as Suzuki's method). In the present study, the distribution of *L. scutellare* larvae was surveyed using the black cloth method along roads passing through hilly and mountainous areas in both prefectures.

MATERIALS AND METHOD

The occurrence of larval trombiculid mites in different vegetation types was surveyed at each of three sites in Kagoshima and Nagasaki Prefectures. In Kagoshima Prefecture, five soil samples each, about 2 liter, were taken at natural woods, grassland, and Japanese cedar farmland in Imakoba (Fukiage Town), Shimotaniguchi (Ijuin Town) and Iwato (Fukuyama Town) on November 1997 (Fig. 1). In Nagasaki Prefecture, five soil samples each were also taken at natural woods and grassland of Kashiya (Nagasaki City), and two sites of Taira (Oseto Town) on December

Fig. 1. Sampling sites (A, Imakoba, Fukiage Town; B, Shimotaniguchi, Ijuin Town; C, Iwato, Fukuyama Town) and routes of sample collection (R1–R5) in Kagoshima Prefecture.

Fig. 2. Sampling sites (A, B, Taira, Oseto Town; C, Kashiya, Nagasaki City) and routes of sample collection (R1–R5) in Nagasaki Prefecture.

1997 (Fig. 2). The natural woods were mainly composed of *Castanopsis cuspidata* var. *sieboldii* and *Quercus salicina* in Kagoshima Prefecture, and *Q. salicina* in Nagasaki Prefecture. The grassland was mainly covered with *Imperata cylindrica* in both prefectures. Soil samples were placed in Tullgren funnels for one week to separate larval trombiculid mites. The mites

Table 1. Occurrence of larval trombiculid mites from soil samples in different vegetation types.

Site	Species	Natural woods	Grassland	Japanese cedar farmland
Kagoshima Prefecture				
Imakoba (Fukiage Town)	<i>Leptotrombidium scutellare</i>	364	146	38
	<i>Leptotrombidium pallidum</i>	4	22	0
	<i>Leptotrombidium himizu</i>	4	0	0
	<i>Leptotrombidium fuji</i>	4	5	10
	<i>Gahrlepiea saduski</i>	0	0	1
Shimotaniguchi (Ijuin Town)	<i>Leptotrombidium scutellare</i>	213	132	193
	<i>Leptotrombidium kuroshio</i>	1	0	0
	<i>Leptotrombidium pallidum</i>	120	15	24
	<i>Leptotrombidium himizu</i>	8	1	1
	<i>Leptotrombidium fuji</i>	7	20	2
	<i>Leptotrombidium kansai</i>	4	0	0
	<i>Walchia ogatai</i>	0	0	4
	<i>Walchia koshikiensis</i>	0	0	1
	<i>Gahrlepiea saduski</i>	8	1	0
	<i>Leptotrombidium scutellare</i>	6	11	6
Iwato (Fukuyama Town)	<i>Leptotrombidium kuroshio</i>	1	0	0
	<i>Leptotrombidium pallidum</i>	3	0	0
	<i>Leptotrombidium himizu</i>	0	0	1
	<i>Leptotrombidium fuji</i>	6	0	2
	<i>Leptotrombidium kansai</i>	1	0	0
	<i>Helenicula miyagawai</i>	0	0	3
	<i>Walchia ogatai</i>	0	0	0
	<i>Walchia koshikiensis</i>	9	0	0
	<i>Gahrlepiea saduski</i>	0	0	0
Nagasaki Prefecture				
Kashiyama (Nagasaki City)	<i>Leptotrombidium scutellare</i>	15	9	—
	<i>Leptotrombidium fukuoka</i>	1	0	—
	<i>Leptotrombidium miyajimai</i>	7	0	—
	<i>Gahrlepiea saduski</i>	1	0	—
Taira A (Oseto Town)	<i>Leptotrombidium scutellare</i>	1	448	—
	<i>Leptotrombidium fukuoka</i>	3	0	—
Taira B (Oseto Town)	<i>Leptotrombidium scutellare</i>	4	12	—
	<i>Leptotrombidium fukuoka</i>	0	1	—

were mounted on glass slides and identified microscopically.

The distribution of *L. scutellare* larvae was surveyed along roads passing through hilly and mountainous areas of Kagoshima and Nagasaki Prefecture. Larval trombiculid mites were collected at intervals of about one kilometer with a black cloth. After each kilometer stop along the routes, larval trombiculid mites were collected in the nearest woods, and the vegetation was also recorded. A black cloth (45×50 cm) was lightly pressed down at several points by hand so as to obtain close contact with the ground or herba-

ceous leaves. Black cloths were placed in the Tullgren funnels for one day to separate larval trombiculid mites. These were mounted on glass slides and identified microscopically.

In Kagoshima Prefecture, surveys were carried out along five routes in December 1997 and 1998: The survey areas were designated R1–R5, respectively. Accordingly, R1=Sukeshiro to Isakutouge, Fukiage Town, 12 sites; R2=Fukuzawa, Fukuyama Town to Tsuneyoshi, Kihoku Town, 11 sites; R3=Tsuneyoshi, Kihoku Town to Nokata, Osaki Town, 7 sites; R4=Tsuneyoshi, Kihoku Town to Iwagawa,

Osumi Town, 7 sites; R5=Shimomyou to Kitayama, Aira Town, 15 sites (Fig. 1). In Nagasaki Prefecture, surveys were also carried out along five routes in December 1998, i.e., R1=Yagiharagou, Seihi Town to Kenminnomori, Kinkai Town, 10 sites; R2=Kenminnomori to Koumonogou, Oseto Town, 10 sites; R3=Saikaigou to Kenminnomori, Kinkai Town, 8 sites; R4=Koba, Chijiwa Town to Unzen, Obama Town, 8 sites; R5=Unzen, Obama Town to Furuemyou, Fukae Town, 7 sites (Fig. 2). To confirm the occurrence of *L. scutellare* larvae, the black cloth collection was also carried out in a woods of *C. cuspidata* var. *sieboldii* in Taira, Oseto Town and Kashiyama, Nagasaki City.

RESULTS

1. Collection of larval trombiculid mites in different vegetation types

In Kagoshima Prefecture, 10 species were collected from soil samples at three sites. *L. scutellare* was dominant, followed by *L. pallidum* and *L. fuji*. *L. scutellare* and *L. pallidum* were collected in three vegetation types. The largest numbers of both species were recorded at the natural woods of two sites and at the grassland of one site (Table 1).

In Nagasaki Prefecture, four species were collected, and *L. scutellare* was also dominant. *L. scutellare* was collected in two vegetation types. The larger number of *L. scutellare* was recorded at the grassland of two sites and at the natural woods of one site (Table 1).

2. Collection of *L. scutellare* larvae along roads

In Kagoshima Prefecture, the black cloth collection of larval trombiculid mites was carried out at 52 sites along five routes, and *L. scutellare* was found in 41 sites (78.8%). Only *L. scutellare* larvae were found in the survey (Table 2). More than ten *L. scutellare* larvae were collected at 14 sites.

In Nagasaki Prefecture, the collection of

larval trombiculid mites was carried out at 43 sites along five routes, and *L. scutellare* was found in 4 sites (9.3%). *L. pallidum*, *L. fuji*, and *Helenicula miyagawai* were found in one site each (Table 3). No site yielded more than one or two larvae. By contrast, 24 and 37 *L. scutellare* larvae were collected at Taira and Kashiyama (control sites), respectively.

DISCUSSION

It was reported that the favorable habitats for *L. scutellare* and *L. pallidum* are well-drained grassland and larger paddy banks exposed to the sun on the slope of Mt. Fuji, Shizuoka Prefecture (Uchikawa et al., 1990). Yabe and Hayashi (1993) suggested that grass-scrub terrain is the ideal habitat for *L. scutellare* in endemic areas in hilly areas of western Kanagawa Prefecture, and a cause of the epidemic of tsutsugamushi disease is the increase of *L. scutellare* along the expanded grass-scrub terrain. Pham et al. (1999) reported that *L. scutellare* was collected at sites that were partially shaded by trees or exposed to the sun, but it was not found at sites that were always shielded from the sun in endemic areas of Oita Prefecture. In contrast to these surveys, the present survey found the largest numbers of *L. scutellare* and *L. pallidum* in the natural woods and grassland in Kagoshima Prefecture, and both species were also collected in the Japanese cedar farmland. In previous surveys carried out in Kagoshima Prefecture, *L. scutellare* and *L. pallidum* were collected at natural woods and Japanese cedar farmlands (Noda et al., 1996; Yamamoto et al., 1997). Therefore, *L. scutellare* and *L. pallidum* seemed to be able to adapt to various habitats, from dry and sunny to wet and shaded. Moreover, *L. scutellare* larvae were also collected from gardens within housing lots and farmlands (Noda et al., 1996). These survey results showed that *L. scutellare* and *L. pallidum* are distributed in various habitats and this distribution seems to relate to the number of pa-

Table 2. *Leptotrombidium scutellare* collected with black cloth along sampling routes in Kagoshima Prefecture.

Sampling site	Sampling route									
	R1		R2		R3		R4		R5	
	Vegetation	No. of mites	Vegetation	No. of mites	Vegetation	No. of mites	Vegetation	No. of mites	Vegetation	No. of mites
1	<i>M. t.</i> , <i>C. c.</i>	211	<i>C. j.</i>	47	<i>Q. s.</i>	2	<i>C. j.</i>	0	<i>C. j.</i>	19
2	<i>C. j.</i>	13	<i>C. j.</i>	37	<i>C. o.</i>	0	<i>C. o.</i>	3	<i>C. j.</i>	0
3	<i>C. j.</i>	5	<i>C. j.</i>	19	<i>C. j.</i>	5	<i>C. j.</i>	0	<i>C. j.</i>	29
4	<i>C. j.</i>	4	<i>C. c.</i>	4	<i>M. t.</i> , <i>Q. s.</i>	13	<i>C. c.</i> , <i>M. t.</i>	9	<i>C. j.</i>	1
5	<i>C. j.</i>	20	<i>Q. s.</i>	6	<i>C. j.</i>	7	<i>C. j.</i>	0	<i>C. j.</i>	64
6	<i>M. t.</i> , <i>C. c.</i>	35	<i>C. j.</i>	0	<i>Q. s.</i> , <i>C. c.</i>	4	<i>C. o.</i>	2	<i>C. o.</i>	6
7	<i>M. t.</i> , <i>C. c.</i>	427	<i>C. j.</i>	3	<i>C. j.</i>	4	<i>C. o.</i>	3	<i>C. j.</i>	4
8	<i>C. c.</i> , <i>Q. s.</i>	0	<i>C. j.</i>	3					<i>M. t.</i> , <i>C. c.</i>	8
9	<i>C. o.</i>	0	<i>C. o.</i>	2					<i>C. j.</i>	9
10	<i>Q. s.</i>	2	<i>M. t.</i>	2					<i>C. o.</i>	0
11	<i>P. p.</i>	62	<i>C. j.</i>	0					<i>C. o.</i>	0
12	<i>C. o.</i>	2							<i>C. j.</i>	21
13									<i>C. j.</i>	6
14									<i>M. t.</i> , <i>C. c.</i>	2
15									<i>M. t.</i> , <i>C. c.</i>	11

M. t., *Machilus thunbergii*; *C. c.*, *Castanopsis cuspidata* var. *sieboldii*; *C. j.*, *Cryptomeria japonica*; *Q. s.*, *Quercus salicina*; *C. o.*, *Chamaecyparis obtusa*; *P. p.*, *Phyllostachys pubescens*.

Table 3. Larval trombiculid mites collected with black cloth along sampling routes in Nagasaki Prefecture.

Sampling site	Sampling route									
	R1		R2		R3		R4		R5	
	Vegetation	No. of mites	Vegetation	No. of mites	Vegetation	No. of mites	Vegetation	No. of mites	Vegetation	No. of mites
1	<i>C. o.</i>	0	<i>C. o.</i>	0	<i>C. c.</i> , <i>M. t.</i>	<i>L. scutellare</i> 2	<i>C. o.</i>	0	<i>P. d.</i> , <i>M. t.</i>	0
2	<i>M. t.</i>	0	<i>C. o.</i>	0	<i>C. c.</i> , <i>Q. s.</i>	0	<i>C. j.</i>	0	<i>C. o.</i>	0
3	<i>C. c.</i> , <i>M. t.</i>	0	<i>C. o.</i>	0	<i>C. o.</i>	<i>L. scutellare</i> 1	<i>C. j.</i>	0	<i>C. o.</i>	0
4	<i>C. c.</i> , <i>Q. s.</i>	0	<i>Q. s.</i> , <i>P. d.</i>	0	<i>C. c.</i> , <i>Q. s.</i>	0	<i>C. j.</i>	<i>H. miyagawai</i> 1	<i>C. o.</i>	0
5	<i>C. c.</i> , <i>Q. s.</i>	<i>L. scutellare</i> 1	<i>C. c.</i> , <i>M. t.</i>	0	<i>C. c.</i> , <i>Q. s.</i>	0	<i>C. c.</i> , <i>M. t.</i>	<i>L. scutellare</i> 1	<i>C. c.</i>	0
6	<i>C. c.</i> , <i>Q. s.</i>	0	<i>M. t.</i> , <i>C. c.</i>	0	<i>C. o.</i>	0	<i>C. j.</i>	0	<i>M. t.</i> , <i>Q. s.</i>	<i>L. pallidum</i> 1
7	<i>Q. s.</i> , <i>C. c.</i>	0	<i>C. c.</i>	0	<i>C. j.</i>	0	<i>C. c.</i>	0	<i>C. o.</i>	0
8	<i>Q. s.</i>	0	<i>C. c.</i> , <i>M. t.</i>	0	<i>C. o.</i>	0	<i>M. t.</i> , <i>C. c.</i>	0		
9	<i>C. o.</i>	0	<i>C. c.</i> , <i>M. t.</i>	<i>L. fuji</i> 2						
10	<i>C. c.</i> , <i>Q. s.</i>	0	<i>C. o.</i>	0						

C. o., *Chamaecyparis obtusa*; *M. t.*, *Machilus thunbergii*; *C. c.*, *Castanopsis cuspidata* var. *sieboldii*; *Q. s.*, *Quercus salicina*; *P. d.*, *Pinus densiflora*; *C. j.*, *Cryptomeria japonica*.

tients in Kagoshima Prefecture.

In our previous surveys carried out in Kagoshima Prefecture, *L. scutellare* larvae were easily found in a variety of habitats. This fact suggests that *L. scutellare* larvae are distributed widely in high density. To confirm this assumption, the distribution of *L. scutellare* larvae was examined using the black cloth method along roads passing through hilly and mountainous areas of Kagoshima and Nagasaki Prefecture. In Kagoshima Prefecture, *L. scutellare* larvae were found at 41 out of 52 sites (78.8%). The black cloth was lightly pressed down at only a few points in the collection of *L. scutellare* larvae. Therefore, there is a possibility that *L. scutellare* larvae are distributed in low density in sites where they were not found in the present survey. The same survey was carried out in Nagasaki Prefecture. There is no great difference in vegetation between Kagoshima and Nagasaki Prefectures, that is, the areas of Kagoshima Prefecture were mainly covered with oak (*Quercus*) and chinquapin (*Castanopsis*), and the areas of Nagasaki Prefecture were mainly covered with chinquapin. However, *L. scutellare* larvae were found at only 4 out of 43 sites (9.3%). In the same week, the occurrence of *L. scutellare* larvae was confirmed at Taira and Kashiya (control sites), Nagasaki Prefecture. Kitazawa (1999) carried out a wide survey of trombiculid mites in northern Kyushu, and suggested that the small number of patients in the region is attributed to the sparse distribution of *L. scutellare*. The wide occurrence and high density of vector mites also seems to relate to the number of patients in Kagoshima Prefecture.

A cluster watching method for sampling *L. scutellare* larvae on a black cloth was first proposed by Suzuki (1986). This method proved to be the most efficient for the sampling of questing *L. scutellare* larvae in the field, and this method was designated as Suzuki's method by Uchikawa et al. (1993). In the present study, the distribution of *L. scutellare* larvae along

roads passing through hilly and mountainous areas was surveyed using Suzuki's method. It may be hard to carry out the same survey by separating *L. scutellare* larvae from soil samples and animals. This method is also the most suitable for collecting a large number of unfed *L. scutellare* larvae.

The occurrence of vector mites for tsutsugamushi disease, especially *L. scutellare*, over a wide area, in high density and in various vegetation types will increase the contact between vector mites and humans, and consequently, the number of patients will increase. The reason why vector mites are abundant in Kagoshima Prefecture requires further study in order to clarify the ecological habits of vector mites. Moreover, the detection of *O. tsutsugamushi* from vector mites is also necessary to understand the epidemiology of tsutsugamushi disease in Kagoshima Prefecture.

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