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Trombiculid fauna and seasonal occurrence of *Leptotrombidium scutellare* and *Leptotrombidium pallidum* in endemic and non-endemic areas for tsutsugamushi disease in Oita Prefecture, Japan

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Abstract: Trombiculid fauna and the seasonal occurrence of its components were surveyed at three fixed sites in an endemic area of tsutsugamushi disease (Taketa City and Asaji Town), and at four fixed sites in a non-endemic area (Oita City and Hasama Town) in Oita Prefecture, Japan. A monthly collection of unengorged larvae was made at each site from December 1996 to January 1998, from soil samples (taken from rodent nest tunnels and from the ground surface without tunnels) using the Tullgren funnel apparatus (Direct method), and/or from the ground surface with a black cloth (Suzuki method). A total of 19 species were identified, including five newly recorded species in Oita Prefecture. *Leptotrombidium scutellare* and *L. pallidum*, which had been suspected as vectors of tsutsugamushi disease in Oita Prefecture, were collected in both endemic and non-endemic areas. The former species seemed to be relatively abundant in the endemic area and very rare in the non-endemic area. By contrast, the latter species seemed to be more abundant in the non-endemic area than in the endemic area. *Leptotrombidium scutellare* larvae occurred from October to January, with a probable peak in November. On the other hand, *L. pallidum* occurred almost throughout the year with two peaks, one in September and the other between November and February. The Direct method, especially for soils of rodent nest tunnels, has been proved satisfactory in studying the fauna and seasonal occurrence of larval trombiculids, while the Suzuki method is more effective in sampling unengorged larvae of *L. scutellare* and *Helenicula miyagawai*.

INTRODUCTION

Since 1984, tsutsugamushi disease caused by *Orientia tsutsugamushi* (Hayashi, 1920), which is now prevalent throughout Japan except Hokkaido, has become one of the important rickettsioses in Oita Prefecture in Kyushu, with 5 to 43 cases in humans reported annually (Ono *et al.*, 1988; Anonymous, 1997). Infections occurred in late autumn and early winter, and a majority of the cases was among the residents of Taketa City and its surrounding towns and villages spread across the northern foothills of the Sobo/Katamuki mountains, a major endemic focus of this disease in Oita Prefecture (Ono *et al.*, 1988).

There have been several reports on trombiculid mites in relation to the transmission of this rickettsiosis in Oita Prefecture (Sasa *et al.*, 1953; Kamo *et al.*, 1955; Miura, 1959; Umeki *et al.*, 1970; Ono *et al.*, 1988). In total, 28 trombiculid species have been recorded by examination of the host rodents or birds, and some strains of *O. tsutsugamushi* were isolated from larvae of five trombiculid species, which had

been removed from wild rodents captured at Hijudai in Beppu City and at Kunisaki Town in Oita Prefecture (Kitaoka *et al.*, 1967; Umeki *et al.*, 1970). The same species of rickettsia was also isolated from *Apodemus speciosus*, captured in four cities, i.e., Bungotakada, Saiki, Beppu and Hita (Tamiya, 1962). Asanuma (1983) reported in his brief review that *Leptotrombidium pallidum* (Nagayo, Miyagawa, Mitamura et Tamiya, 1919) is a vector for tsutsugamushi disease in Taketa City, based on the isolation of *O. tsutsugamushi* from this species of mite collected in this city. Ono *et al.* (1988) carried out an extensive survey on trombiculid mites on rodents, and suggested the importance of *L. pallidum* and *L. scutellare* (Nagayo, Miyagawa, Mitamura, Tamiya et Tenjin, 1921) in the transmission of this disease in Oita Prefecture. However, no work on the seasonal occurrence of these two species in and around Taketa City has hitherto been done.

The purpose of our study was to determine the vector for this disease in Taketa City by investigating the fauna and seasonal occurrence of larval trombiculid mites, especially of *L. pallidum* and *L. scutellare*, and by detecting the causative rickettsial agent in unengorged individuals of either or both of these trombiculids. Therefore, we attempted to collect trombiculid larvae from soil samples in nest tunnels of rodents and on the ground surface, using the Tullgren funnel apparatus (Suzuki, 1973, 1977; called the Direct method by Suzuki, 1980), and also from the ground surface using a black cloth (Suzuki and Tabaru, 1987; designated as the Suzuki method by Uchikawa *et al.*, 1996).

In this paper, we report the results of this study on the fauna and seasonal occurrence of trombiculid mites in an endemic area (Taketa City and Asaji Town) and in a non-endemic area (Oita City and Hasama Town). The usefulness of the respective methods in collecting unengorged larvae is emphasized.

Survey Sites and Methods

Endemic area. We selected two sites at Naka and Shimohirata in Taketa City, and one site at Jinkakuji Valley in Asaji Town to survey the fauna and seasonal occurrence of larval trombiculid mites (Fig. 1). Site 1 was in Naka, in the western part of the city. The surveyed area was covered with low clumps of grasses and bamboo, and was partially shaded by oak and chestnut trees. Site 2 was in Shimohirata, in a basin of the Inaba River, a tributary of the Ohno River, near the center of Taketa City. This site was in a chestnut orchard, halfway up the slope of Shimohirata at an altitude of ca. 200 m. It was exposed to the sun, and covered with low grasses. There were many terraced paddies around this site. Site 3 was in Jinkakuji Valley, Asaji Town, where a rapid stream (ca. 10 m wide) runs through a coniferous forest. No dwellings exist. The site was near a narrow path along the stream, and was always shielded from the sun.

Non-endemic area. Two sites (Kobaru and Toi) in Oita City, and two sites (Idaigaoka and Miyaura) in Hasama Town were selected to survey the fauna and seasonal occurrence of larval trombiculid mites (Fig. 1). Site 4 was in a small, isolated, mixed forest of bamboo and tall evergreen trees on an abandoned knoll, close to Oita Medical University in Idaigaoka, Hasama Town. The floor was covered with fallen bamboo leaves and shaded by a canopy of trees. Site 5 was in a small forest with several tall deciduous trees in Kobaru, Oita City, the floor of which was covered with low grasses, and was partially exposed to the sun. Site 6 was located in a small forest with bamboo and chestnut trees in Toi, Oita City, with its floor covered with fallen tree leaves and rotten twigs, and always shielded from the sun. Site 7 was in a forest in Miyaura in Hasama Town. It was predominantly shaded by bamboo and a few tall trees. The floor was covered with fallen tree leaves, twigs,

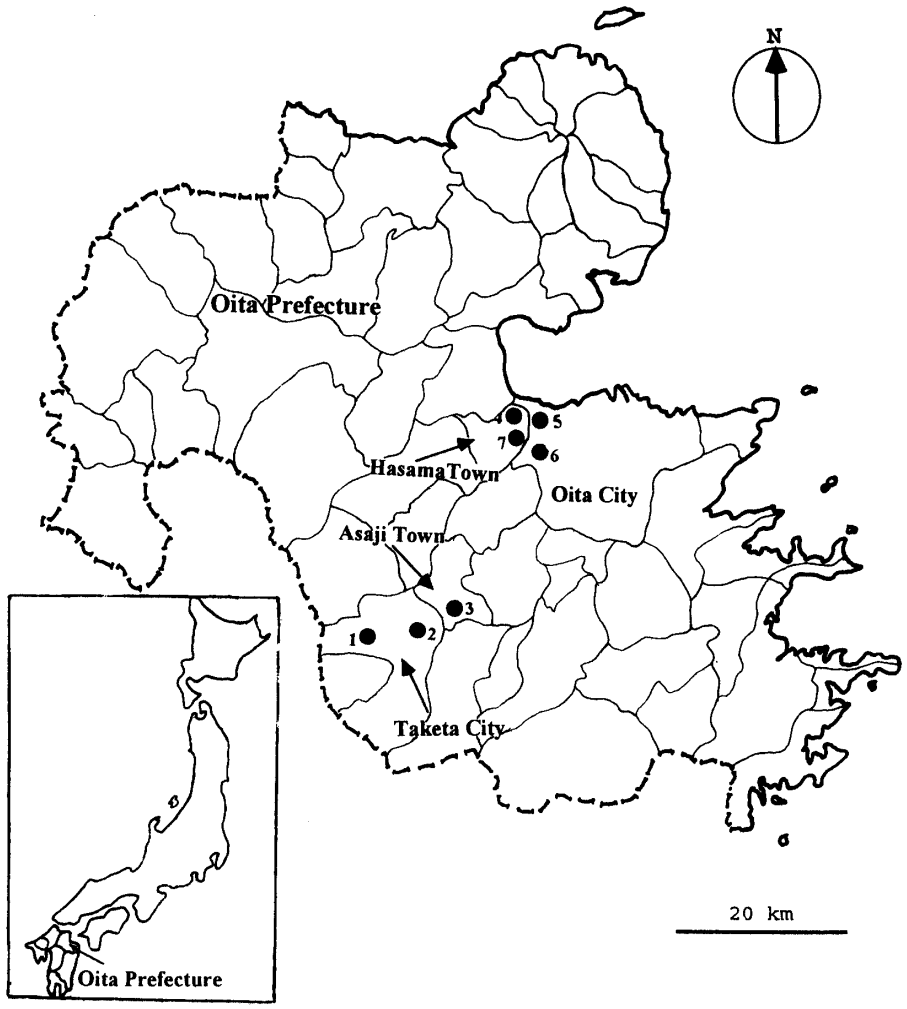

Fig. 1. Map of Oita Prefecture showing seven sites where monthly collections of trombiculid mites were carried out.
Bottom left: Map of Japan showing location of Oita Prefecture.

and decaying wood. Surrounding this forest were cultivated fields, both rice paddies and vegetable fields.

Four soil samples (approximately 1 kg per sample) were collected at each fixed site: i.e., one was collected at or near the entrance of rodent nest tunnels (Suzuki, 1973, 1977), three others randomly from the ground surface without tunnels of field rodents (hereafter called free soil samples). Each free soil sample was dug out in a size of 20 cm square × 5 cm deep at the selected sites (Uchikawa *et al.*, 1986). Soil and litter samples were maintained in separate plastic bags, and trombiculid mites were recovered using the Tullgren funnel apparatus in the laboratory as reported by Suzuki (1973). Sampling was made from December 1996 to January 1998.

Additional surveys were carried out at 16 sites (five sites in Taketa City, three sites in Asaji Town, one site in Hasama Town and seven sites in Oita City) to supplement the data on the fauna of trombiculid mites. Approximately 1 kg of soil and litter was gathered in plastic bags from the nest tunnels of rodents at each site for the isolation of mites by the Tullgren funnel apparatus.

In addition, the Suzuki method was used in monthly samplings at site 1 and site 2, in order to obtain unengorged larvae of *L. scutellare* or other trombiculid mite species, which may breed in clusters on the ground surface. This method was also

applied at sites 5, 6 and 7 from October to December 1997, and at some other unfixed sites. Larval trombiculid mites on the ground surface of the surveyed sites (ca. 2 m×2 m quadrate) were allowed to move on a 45 cm×45 cm quadrate black cloth. A black cloth spread on the quadrate was pressed down lightly with the collector's palm, so as to achieve close contact with the ground or herbaceous leaves. Larval trombiculid mites on the underside of the cloth were individually picked up using the tip of a bamboo leaf and transferred into vials with 80% ethanol solution.

Results

Trombiculid fauna of the two areas surveyed. In total 3,788 larval trombiculid mites were collected, and classified into 19 species of 10 genera, of which 17 species were obtained only by the Direct method; one species, i.e., *Helenicula miyagawai* (Sasa, Kumada et Miura, 1951), only by the Suzuki method; and one species, i.e., *L. scutellare*, by both methods (though many more individual mites were collected by the latter method) (Table 1). At all sites, most species collected were more numerous in nest tunnel soil samples than in free soil samples (Table 2). Five species were newly recorded in Oita Prefecture, i.e., *Doloisia* sp. (the same as reported from Kagoshima Prefecture by Suzuki *et al.*, in 1996), *H. miyagawai*, *L. himizu* (Sasa, Kumada, Hayashi, Enomoto, Fukuzumi et Obata, 1951), *Neoschoengastia shiraii* Sasa et Sato, 1953 and *Walchia ogatai* Sasa et Teramura, 1951.

In the endemic area (Taketa City and Asaji Town) 17 species were obtained (Table 1). *L. kitasatoi* (Fukuzumi et Obata, 1950) and *L. fuji* (Kuwata, Berge et Philip, 1950) were relatively dominant at all three fixed sites, but *L. scutellare* was collected in substantial numbers only at sites

Table 1. Species and numbers of unengorged larval trombiculid mites collected by the Direct method and the Suzuki method at six fixed sites and sixteen other sites.

Species	Taketa and Asaji			Oita and Hasama		
	A	B	C	A	B	C
Cheladonta ikaoensis	2	0	0	0	0	0
Doloisia sp.	0	0	0	17	7	0
Euschoengastia alpina	5	0	0	1	0	0
Gahrliepia saduski	32	5	0	154	53	0
Helenicula miyagawai	0	0	165	0	0	0
Leptotrombidium fuji	95	33	0	217	55	0
L. himizu	14	17	0	56	14	0
L. kansai	10	0	0	0	0	0
L. kitasatoi	318	36	0	853	257	0
L. kuroshio	0	0	0	12	0	0
L. miyajimai	29	4	0	3	0	0
L. murotoense	2	0	0	0	0	0
L. pallidum	112	12	0	440	135	0
L. scutellare	56	16	368	9	1	15
L. tanakaryoi	22	9	0	3	0	0
Miyatrombicula kochiensis	17	2	0	26	18	0
Neoschoengastia shiraii	5	1	0	1	0	0
Neotrombicula japonica	18	0	0	0	0	0
Walchia ogatai	11	5	0	19	1	0
Total	748	140	533	1,811	541	15

A, No. of mites isolated from samples of nest tunnel soil; B, No. of mites isolated from samples of free soil; C, No. of mites collected by the Suzuki method.

Table 2. The total numbers of unengorged larval trombiculid mites recovered by the Direct method from one sample of nest soil and three samples of free soil*.

Fixed site Mite species	A	B
Site 1		
G. saduski	2	0
L. fuji	17	10
L. kitasatoi	36	12
L. pallidum	5	1
L. scutellare	17	4
M. kochiensis	1	0
W. ogatai	1	0
Site 2		
E. alpina	1	0
G. saduski	1	2
L. fuji	19	11
L. himizu	5	16
L. kansai	1	0
L. kitasatoi	54	7
L. scutellare	37	12
M. kochiensis	5	2
W. ogatai	1	0
Site 3		
G. saduski	14	3
L. fuji	16	12
L. himizu	1	1
L. kansai	3	0
L. kitasatoi	19	17
L. miyajimai	21	4
L. pallidum	26	11
L. tanakaryoi	19	9
M. kochiensis	3	0
N. shiraii	5	1
W. ogatai	5	5
Site 4		
G. saduski	31	5
L. fuji	80	12
L. himizu	3	4
L. kitasatoi	5	4
L. pallidum	228	71
W. ogatai	4	0
Site 5		
Doloisia sp.	17	7
G. saduski	37	31
L. fuji	25	3
L. himizu	33	10
L. kitasatoi	94	76
L. pallidum	58	27
L. scutellare	7	1
M. kochiensis	2	0
W. ogatai	6	1
Site 6		
G. saduski	19	12
L. fuji	46	28
L. himizu	2	0
L. kitasatoi	436	125
L. kuroshio	5	0
L. miyajimai	1	0
L. pallidum	33	15
M. kochiensis	7	16
Site 7		
G. saduski	10	5
L. fuji	26	12
L. himizu	1	0
L. kitasatoi	143	52
L. kuroshio	3	0
L. miyajimai	2	0
L. pallidum	33	22
M. kochiensis	2	2

A, No. of mites from monthly collections of 1 sample of nest tunnel soil; B, No. of mites from monthly collections of 3 samples of free soil.

* Nest soil and free soil samples were taken monthly at site 5 from March 1997 and at six other fixed sites from February 1997 to January 1998. Free soil samples were not taken at site 5 in February 1997.

1 and 2, and *L. pallidum* only at site 3 (Table 2). On the other hand, 14 species were obtained in the non-endemic area (Oita City and Hasama Town) (Table 1). Here *L. kitasatoi* and *L. fuji* were also dominant at three or four fixed sites. In addition, *L. pallidum* and *Gahrliepia saduski* Womersley, 1952 were relatively abundant at all four fixed sites. By contrast, *L. scutellare* was rare, only eight larvae being collected at site 5 (Table 2).

Seasonal occurrence of L. scutellare *and* L. pallidum. In the endemic area, 72 and 368 *L. scutellare* were collected at two fixed sites in the Taketa/Asaji area by the Direct and the Suzuki methods, respec-

tively. This species was captured from October to December by the Direct method, and from October to January by the Suzuki method, respectively, and was most abundant in November by both methods. On the other hand, only 37 *L. pallidum* were collected in this endemic area. They were found from August to January and in May (Table 3).

In the Oita/Hasama area, all eight *L. scutellare* larvae were found in November. On the other hand, a total of 409 and 135 *L. pallidum* were collected from nest tunnel and free soil samples, respectively. Larvae of *L. pallidum* were detected in nest tunnel soil samples throughout the year except in June, and seemed to be relatively abundant in September and in

Table 3. Results of monthly collections of unengorged larvae of *Leptotrombidium scutellare* and *L. pallidum* at three fixed sites in the Taketa/Asaji endemic area of tsutsugamushi disease.

Year	1996	1997											1998
Month	12	1	2	3	4	5	6	7	8	9	10	11	12	1
Site 1 (*L. scutellare*)														
A	0	0	0	0	0	0	0	0	0	0	4	10	3	0
B	ND	ND	0	0	0	0	0	0	0	0	0	4	0	0
C	30	2	0	0	0	0	0	0	0	0	8	201	4	0
Site 2 (*L. scutellare*)														
A	2	0	0	0	0	0	0	0	0	0	5	31	1	0
B	ND	ND	0	0	0	0	0	0	0	0	0	12	0	0
C	54	0	0	0	0	0	0	0	0	0	2	65	2	0
Site 3 (*L. pallidum*)														
A	ND	ND	0	0	0	2	0	0	5	7	3	4	4	1
B	ND	ND	0	0	0	0	0	0	4	2	3	2	0	0

A, No. of mites isolated from 1 sample of nest tunnel soil; B, No. of mites isolated from 3 samples of free soil; C, No. of mites collected by the Suzuki method.
ND, Not done.

Table 4. Results of monthly collections of unengorged larvae of *Leptotrombidium pallidum* at four fixed sites in Oita City and Hasama Town, non-endemic for tsutsugamushi disease.

Year	1997												1998
Month	1	2	3	4	5	6	7	8	9	10	11	12	1
Site 4													
A	37	46	0	0	2	0	6	5	14	5	8	120	22
B	ND	10	0	0	0	0	1	1	17	0	6	31	5
Site 5													
A	9	1	0	0	0	0	0	0	6	1	15	23	13
B	ND	ND	0	0	0	0	0	0	4	1	5	3	14
Site 6													
A	5	0	0	0	0	0	0	2	11	4	2	10	4
B	ND	0	0	0	0	0	0	2	3	0	0	8	2
Site 7													
A	5	0	2	1	0	0	0	5	6	0	1	3	15
B	ND	0	0	0	0	0	0	5	6	0	0	0	11

A, No. of mites isolated from 1 sample of nest tunnel soil; B, No. of mites isolated from 3 samples of free soil.
ND, Not done.

Table 5. Results of monthly collections of unengorged larval trombiculid mites of three common species (i.e., *G. saduski*, *L. fuji*, *L. kitasatoi*) recovered from soil samples taken from seven fixed sites in Oita Prefecture.

Year	1997												1998
Month	1	2	3	4	5	6	7	8	9	10	11	12	1
G. saduski													
A	30	35	2	1	13	6	1	0	0	2	16	23	15
B	ND	7	0	0	2	1	0	0	0	2	20	11	15
L. fuji													
A	25	49	2	5	4	1	3	40	30	18	9	43	25
B	ND	14	4	3	2	0	1	15	23	4	12	2	8
L. kitasatoi													
A	79	86	4	36	7	12	17	99	46	85	54	139	202
B	ND	3	2	29	1	6	2	18	19	27	47	60	79

A, No. of mites isolated from nest tunnel soil; B, No. of mites isolated from free soil.
ND, Not done.

the period from November to February (Table 4).

Three other common species, i.e., *L. kitasatoi*, *L. fuji* and *G. saduski* occurred almostyear-roundthough the last species was not found in August and September (Table 5).

Discussion

For many years, investigations on the vectors of scrub typhus have been carried out by collecting larval trombiculid mites from host rodents and birds in the field. Suzuki (1973, 1977) introduced an alternative method for collecting unengorged larvae of trombiculid mites directly from the soil of rodent nest tunnels with the Tullgren funnel apparatus, which was designated the Direct method by Suzuki (1980). Suzuki (1978, 1980, 1981) proved this new method to be much simpler and more efficient than the conventional host-capture method. Uchikawa *et al.* (1986) showed the effectiveness of this Direct method to detect unengorged *L. pallidum* larvae not only from soils of rodent nest holes but also from those of ground surface. They stated that this new method gave better epidemiological information on the risk of human infection with *O. tsutsugamushi*. Again, in their review, Uchikawa *et al.* (1996), pointing out several problems or drawbacks of the host-capture method, recommended the Direct method for sampling vector chiggers. Nevertheless, the Direct method has been so far employed only by a few investigators, e.g., Kitazawa (1993) for trombiculid fauna in Fukuoka Prefecture, Yamamoto and Noda (1995) and Noda *et al.* (1996) for the fauna and the seasonal occurrence of trombiculid mites in Kagoshima Prefecture. We used this method for the first time to survey the fauna of trombiculid mites in Oita Prefecture, and uncovered as many as 18 species, including four newly recorded in this prefecture. The substantial number of unengorged larvae found with this Direct method, especially those isolated from the soil of rodent nest tunnels, allowed us to determine the seasonal occurrence of several common mite species, including *L. pallidum* and *L. scutellare*, although the Suzuki method was more effective than the Direct method in obtaining the latter species (Table 3), as previously reported by Suzuki and Tabaru (1987) and Uchikawa *et al.* (1996). The usefulness of the Suzuki method has also been proved for *H. miyagawai*, which is also newly recorded in Oita (Table 1). It is also noteworthy that unengorged larvae of as many as 12 trombiculid mite species, in-

cluding *L. pallidum* and *L. scutellare*, were collected also from free soil samples, indicating a much wider distribution range of unengorged larvae than might be expected (i.e., at or near rodent nests) (Table 2). However, three samples of free soils per each site per month as taken in this study were inadequate to observe the seasonal occurrence of unengorged larvae of these two species (Tables 3 and 4).

Overall, five species are newly recorded in this study bringing the total number of trombiculid mite species in Oita to 33.

Almost the same number of trombiculid species (i.e., 17 and 14 spp.) was collected in the endemic and the non-endemic areas. However, there were differences in species composition and in the relative abundance of trombiculid mites between the two areas (Table 1), and also between the sites within the same area. This might be a reflection of the topographical and ecological differences in the environment of the selected sites. One such example is *L. scutellare*, which seems to avoid sites with a thick covering of fallen leaves on the ground surface (e.g., sites 3–7), as observed by Wada *et al.* (1992).

Both *L. scutellare* and *L. pallidum* are well-known vectors of tsutsugamushi disease in many places in Japan (Kawamura *et al.*, 1995). In this study, both species were found in the endemic area, as well as in the non-endemic area. However, it should be noted that *L. scutellare* seems to be relatively abundant in the endemic area and very rare in the non-endemic area. By contrast, *L. pallidum* seems to be more abundant in the non-endemic area than in the endemic area.

Our monthly collections indicate that *L. scutellare* larvae occur from October to January, with a probable peak in November (Table 3). A similar pattern of limited seasonal occurrence of the same species has been already shown elsewhere (e.g., in Yamakita Town, Kanagawa Prefecture by Uchikawa *et al.*, 1994, and in Kagoshima Prefecture by Noda *et al.*, 1996).

On the other hand, *L. pallidum* occurs almost throughout the year with two peaks, one in September and the other between November and February (Table 4), though the pattern is only partially substantiated in the endemic area (only site 3) (Table 3). Our results for *L. pallidum* are almost the same as those reported in Kagoshima Prefecture by Noda *et al.* (1996). Such a prolonged seasonal occurrence has been previously suggested by Ono *et al.* (1988), who collected *L. pallidum* larvae by monthly examination of host rodents during the surveyed period from November to April at Jumonjibaru, Beppu City, one of the other endemic areas in Oita Prefecture. The difference in larval occurrence between *L. scutellare* and *L. pallidum* may be explained by the different tolerance of unengorged larvae of both mite species against the coldness of winter, as already reported by Takahashi *et al.* (1993, 1994).

In relation to the transmission of tsutsugamushi disease in the Taketa/Asaji endemic area, our data may suggest the difference in the vectorial importance between *L. scutellare* and *L. pallidum*. Particular attention should be focused on the former species since the seasonal occurrence of human infection with *O. tsutsugamushi* in this endemic area correlates well with the peak seasonal activity of *L. scutellare* larvae. It is likely, however, that vector species differ by localities, since the relative abundance of both species is different from one place to another, even within the endemic area (Table 2). Moreover, information on rickettsial agents infecting both humans and unengorged trombiculids is indispensable in determining the role played by each possible vector.

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摘　　要

大分県におけるツツガムシの種類相およびツツガムシ病の流行地および非流行地でのタテツツガムシとフトゲツツガムシの季節消長

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大分県におけるツツガムシの種類相と季節消長の調査を，ツツガムシ病流行地の 3 地点，非流行地の 4 地点を主な調査地として実施した．各地点で，小哺乳類の坑道と坑道以外の地表からそれぞれ土壌を採取し，ツルグレン装置で未吸着幼虫を抽出した（直接採集法）．また地表を黒布で覆って採集する方法（鈴木の方法）も併用した．その結果 19 種のツツガムシが得られ，そのうちの 5 種が大分県での初記録であった．タテツツガムシとフトゲツツガムシは，どちらも流行地と非流行地の双方から採集されたが，前者が流行地に比較的多く，非流行地には少なかったのに対し，後者は流行地より非流行地に多かった．タテツツガムシの幼虫は 11 月をピークとして 10 月から 1 月に現われたが，フトゲツツガムシは 9 月と，11 月から 1 月にピークをもつ二峰性を示してほぼ一年中現われた．直接採集法はツツガムシ相や季節的消長の調査に効果的に適用できるが，タテツツガムシとミヤガワタマツツガムシの採集には，鈴木の方法が優れていた．