# DISTRIBUTION OF UNENGORGED LARVAE OF *LEPTOTROMBIDIUM PALLIDUM* AND OTHER SPECIES IN AND AROUND THE RODENT NEST HOLES

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**Abstract.** The distribution of unengorged larvae of *Leptotrombidium pallidum*, *L. fuji* and *L. kitasatoi* in and around 12 rodent-nest holes in Oita Prefecture, Japan was studied using the Tullgren funnel apparatus. Soil was taken from each nest hole, and the ground-surface soil and litter from the surrounding area A (an inner quadrate of 20 cm x 20 cm except the nest hole), and also from the outer area B (an outer quadrate of 40 cm x 40 cm excluding A and the nest hole) were sampled, separately. The numbers (% of the total) of *L. pallidum* collected from soil samples of the nest holes and areas A and B were 38 (19.0), 111 (55.5) and 30 (15.0); those of *L. fuji* were 171 (58.8), 104 (35.7) and 14 (4.8); those of *L. kitasatoi* were 35 (77.9), 7 (15.6) and 3 (6.7), and those of *G. saduski* were 20 (50.0), 17 (42.5) and 3 (7.5). The larvae recovered from litter samples were few, representing 0-8.5% of the total. It is shown that unengorged larvae of these species are distributed not only in the nest holes but also in the nearby areas, and exist mainly on (or in) the soil.

## INTRODUCTION

Certain trombiculid mite species transmit *Orientia tsutsugamushi* (Hayashi, 1920), a causative agent of tsutsugamushi disease, to humans through the bite in the larval stage (Kawamura *et al*, 1995).

The sampling methods for larvae of trombiculid mites have been well studied. The host-capture method has been used for a long time and has proven effective in the collection of many trombiculid species (Uchikawa *et al*, 1996). However, the trombiculid mites obtained showed only that they had been living within the home ranges of respective host rodents and did not exactly indicate their micro-habitats in nature. Furthermore, the mites collected by the rodent-capture method are in the course of feeding, then this method is not advantageous for the purpose of studying the pathogen in order to determine the vectors and/or estimate the epidemiological risk of tsutsugamushi disease. This is because trombiculid mites feed only once on an animal host, or on humans, in the larval stage. They can ingest *O. tsutsugamushi* from an infected host during the feeding process (Toyokawa 1972; Traub *et al*, 1975; Walker *et al*, 1975; Takahashi *et al*, 1990, 1994). However, engorged larvae collected from animal hosts do not have opportunity to transmit *O. tsutsugamushi* (originating from either their mother mites or animal hosts) to humans, since they do not feed again. Subsequently, Suzuki (1973, 1977) introduced a method for collecting larvae directly from the soil of rodent nest holes with the Tullgren funnel apparatus (it was called the "Direct method" by Suzuki, 1981). The method was shown to be much simpler and more efficient than the conventional host-capture method. This method also proved to be effective in obtaining *Leptotrombidium pallidum* (Nagayo, Miyagawa, Mitamura et Tamiya, 1919) and *Leptotrombidium scutellare* (Nagayo, Miyagawa, Mitamura, Tamiya et Tenjin, 1921), two important vectors of tsutsugamushi disease in Japan (Suzuki, 1978; 1980, 1981; Uchikawa *et al*, 1986, 1996, Noda *et al*, 1996). Later, Suzuki and Tabaru (1987) reported another new method for collecting unengorged larvae of *L. scutellare* from

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the ground surface using a black cloth (it was called the "Suzuki method" by Uchikawa *et al*, 1996). This new method was much more effective in obtaining *L. scutellare* than the Direct method (Uchikawa *et al*, 1994, 1996; Noda *et al*, 1996; Pham *et al*, 1999), though it is not applicable in collecting *L. pallidum*.

The Direct method is usually used to isolate the larvae of *L. pallidum* and other trombiculid species from the soil of rodent nest holes (Suzuki *et al*, 1973, 1977). Uchikawa *et al* (1986) showed that *L. pallidum* could be obtained not only from the soil of rodent nest holes but also from that of the ground surface by the Direct method.

In order to develop an effective method for collecting the unengorged larvae of *L. pallidum* and some other chigger species from soil, we examined their distribution of unengorged larvae in and around rodent nest holes.

## METHODS

The survey was carried out at three locations in Oita Prefecture; *ie*, Idaigaoka (6 experiments; *ie*, I-1, I-2, I-3, I-4, I-5 and I-6) in Hasama Town; Kobaru (4 experements; *ie*, K-1, K-2, K-3 and K-4) and Toi (2 experements; *ie*, T-1 and T-2) in Oita City, where *L. pallidum* was previously found to be abundant. The collection sites have been previously described in detail (Pham *et al*, 1999). Briefly, Idaigaoka is a small, isolated, mixed forest of bamboo and tall evergreen trees on an abandoned knoll. Kobaru is a small forest of tall deciduous trees. Toi is located in a small forest of bamboo and chestnut trees. The ecological conditions of these sites examined were as follows: the ground of T-1 and T-2 was covered by litter, and the soil was wet. I-1, I-2, I-3, I-4 and I-6 were covered by decaying wood and litter; the soil was humid. I-5 was a grassland and dry soil square. K-1 and K-2 were thickly covered by litter, and the soil was humid. K-3 and K-4 were thinly covered by litter, and the soil was dry.

We selected a 60 cm quadrate of ground surface, with only one rodent nest hole in the center. Three soil and two litter samples were taken from this quadrate in the following manner. From the surrounding area A (an inner quadrate of 20 cm x 20 cm except for the nest hole), two samples (one for soil and one for litter) were taken separately (they were called soil A and litter A). The soil was sampled by digging out a section 5 cm deep of all the ground surface of area A. From the outer area B (an outer quadrate of 40 cm x 40 cm, excluding area A and the nest hole), two samples were collected separately in the same way (they were called soil B and litter B). Finally, one more sample was taken by digging out the tunnel wall of the nest hole with a size of about 3 cm thick and 15 cm deep (it was called "tunnel") (Fig 1).

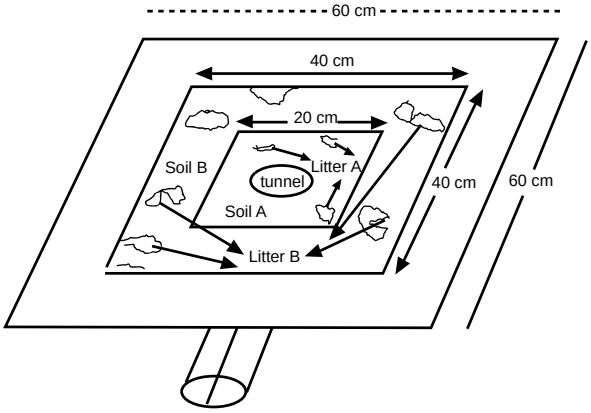


Fig 1–The schema of a rodent nest hole and its vicinity showing the site or areas from where soil and litter samples were taken.

All soil and litter samples were separately maintained in plastic bags. Then unengorged larvae of trombiculid mites were recovered by the Tullgren funnel apparatus in our laboratory. These samplings were made from December 1998 to February 1999.

## RESULTS

In total, 586 unengorged larvae of seven trombiculid species were obtained from 12 sites of rodent nest holes. *ie*, *L. pallidum*, *Leptotrombidium fuji* (Kuwata, Berge et Philip, 1950), *Leptotrombidium kitasatoi* (Fukuzumi et Obata, 1950), *Gahrliepia saduski* Womersley, 1952,

*Miyatrombicula kochiensis* (Sasa, Kawashima et Egashira, 1952), *Walchia ogatai* Sasa et Teramura, 1951 and *Doloisia* sp (same as reported from Kagoshima Prefecture by Suzuki *et al* in 1996). Of those, the first four species were relatively abundant. In all, 46.7% larvae were collected from the soil of tunnels, 40.9% from soil A, 8.5% from soil B, 3.2% from litter A and 0.7% from the litter B (Table 1).

*L. pallidum* was collected from 9 of 12 sites surveyed. At 8 of 9 positive sites the number of *L. pallidum* larvae in soil A was equal to, or larger than, that in the tunnel. Only at site K-1, where 7 larvae were collected, this number was larger in the tunnel than in soil A. In total, 55.5% of *L. pallidum* were isolated from soil A, followed by 19.0% from the tunnels (Table 2). By contrast, *L. fuji* was obtained from 10 of 12 sites surveyed. The larvae of this species were most numerous in the nest tunnel at all but 2 sites (I-2, I-6) (Table 3). A similar result was obtained for *L. kitasatoi* and *G. saduski*.

## DISCUSSION

Our results show that unengorged larvae

Table 1
Species and numbers of unengorged larval chigger mites collected from 12 rodent nest holes by the Direct method.

| Chigger species[a] | Tunnel No. (%) | Soil A No. (%) | Litter A No. (%) | Soil B No. (%) | Litter B No. (%) | Total |
|---|---|---|---|---|---|---|
| *L. pallidum* | 38 (19.0) | 111 (55.5) | 17 (8.5) | 30 (15.0) | 4 (2.0) | 200 |
| *L. fuji* | 171 (58.8) | 104 (35.7) | 2 (0.7) | 14 (4.8) | 0 (0.0) | 291 |
| *L. kitasatoi* | 35 (77.9) | 7 (15.6) | 0 (0.0) | 3 (6.7) | 0 (0.0) | 45 |
| *G. saduski* | 20 (51.3) | 16 (41.0) | 0 (0.0) | 3 (7.7) | 0 (0.0) | 39 |
| *M. kochiensis* | 4 (66.7) | 2 (33.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 6 |
| *W. ogatai* | 4 (100) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 |
| *Doloisia* sp[b] | 1 (100) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 |
| Total (%) | 273 (46.7) | 240 (40.9) | 19 (3.2) | 50 (8.5) | 4 (0.7) | 586 |

[a]L. *Leptotrombidium*; G. *Gahrliepia*; M. *Miyatrombicula*; W. *Walchia*.
[b]As reported by Suzuki *et al* (1996).

Table 2
Numbers of *Leptotrombidium pallidum* obtained from litter and soil by the Direct method at 12 rodent nest holes.

| Location | Tunnel | Soil A | Litter A | Soil B | Litter B | Total |
|---|---|---|---|---|---|---|
| T-1 | 0 | 0 | 0 | 0 | 0 | 0 |
| T-2 | 0 | 0 | 0 | 0 | 0 | 0 |
| I-1 | 7 | 10 | 0 | 6 | 0 | 23 |
| I-2[a] | 7 | 49 | 12 | 13 | 0 | 81 |
| I-3 | 9 | 20 | 0 | 3 | 0 | 32 |
| I-4 | 3 | 10 | 2 | 4 | 2 | 21 |
| I-5 | 1 | 3 | 0 | 1 | 0 | 5 |
| I-6 | 1 | 14 | 1 | 2 | 2 | 20 |
| K-1 | 5 | 0 | 1 | 1 | 0 | 7 |
| K-2 | 4 | 4 | 1 | 0 | 0 | 9 |
| K-3 | 1 | 1 | 0 | 0 | 0 | 2 |
| K-4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total (%) | 38(19.0) | 111(55.5) | 17(8.5) | 30(15.0) | 4(2.0) | 200(100) |

[a]A rat was caught by Sherman trap at this site.

Table 3
Numbers of *Leptotrombidium fuji* recovered from litter and soil by the direct method at 12 rodent nest holes.

| Location | Tunnel | Soil A | Litter A | Soil B | Litter B | Total |
|---|---|---|---|---|---|---|
| T-1 | 18 | 1 | 0 | 0 | 0 | 19 |
| T-2 | 5 | 2 | 0 | 0 | 0 | 7 |
| I-1 | 54 | 4 | 0 | 4 | 0 | 62 |
| I-2[a] | 49 | 78 | 2 | 2 | 0 | 131 |
| I-3 | 10 | 7 | 0 | 4 | 0 | 21 |
| I-4 | 8 | 5 | 0 | 3 | 0 | 16 |
| I-5 | 2 | 1 | 0 | 1 | 0 | 4 |
| I-6 | 0 | 1 | 0 | 0 | 0 | 1 |
| K-1 | 0 | 0 | 0 | 0 | 0 | 0 |
| K-2 | 19 | 5 | 0 | 0 | 0 | 24 |
| K-3 | 6 | 0 | 0 | 0 | 0 | 6 |
| K-4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total (%) | 171(58.8) | 104(35.7) | 2(0.7) | 14(4.8) | 0(0.0) | 291(100) |

[a]A rat was caught by Sherman trap at this site.

of *L. pallidum* on/in the soil of the immediate vicinity are more numerous than those in the tunnels of rodent nest holes while the larvae of other species, *ie*, *L. fuji*, *L. kitasatoi*, *G. saduski*, *M. kochiensis*, *W. ogatai* and *Doloisia* sp, are more numerous in the tunnel. *L. pallidum* seems to be a surface dweller and to be more widely distributed on the ground surface than the other species, since as many as 80% of larvae of this species were obtained outside the tunnel (Table 1). Our present result coincides with that of Nadchatram (1970) who reported that the orange to red colored trombiculid larvae are ground surface dwellers, while the white to yellow ones are adaptive to underground environments. Among the seven species collected in our survey, *L. pallidum* larvae have the reddest body color.

In this study, 10.5% of *L. pallidum* collected were obtained from litter, indicating that most larvae of this species remain in/on the soil. This might be one of the reasons why the Suzuki method could not collect them.

The number of unengorged larvae collected greatly differed by sites. These differences might be caused by several environmental factors. The density of litter and humidity might have influenced the larval population of *L. pallidum* and some other species. In fact, when the ground was too thickly covered by litter and contained high humidity, the number of larvae was reduced (*eg*, T-1 and T-2). Sites, where the soil was too dry, also showed a small number of larvae (*eg*, I-5, K-3 and K-4). Uchikawa *et al* (1994) indicated that humid places obscured by trees and/or covered with grasses were more likely to yield more larvae than dry places. On the other hand, *G. saduski* larvae seem to have adjusted themselves to dry places. This species might have adapted to a deep underground environment.

The presence or absence of rodents in the holes also one of the important factors determining the number of larvae. A rat was caught at I-2 with a Sherman trap just before we took soil and litter samples. As many as 231 larvae were collected from this site.

In conclusion, our study shows that a considerable number of unengorged larvae of *L. pallidum* exist on (or in) the soil not only in the nest hole but also in its immediate vicinity. The distribution pattern in and around the nest holes differs between *L. pallidum* and other species (*ie*, *L. fuji*, *L. kitasatoi*, and *G. saduski*). Soil samples should be taken from nest holes and also from their immediate vicinities in order to collect as many unengorged larvae of *L. pallidum* as possible from the soil.

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