A NEW MEMBER OF THE TROMBICULID MITE FAMILY *NEOTROMBICULA NAGAYOI* (ACARI: TROMBICULIDAE) INDUCES HUMAN DERMATITIS

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Abstract. We present the first definitive evidence that the mite *Neotrombicula nagayoi* bites humans under natural conditions in Japan. Initially, bites resulted in mild pruritus without pain. However, skin reactions increased gradually year by year with severe pruritus with pain being reported by the victim after being bitten repeatedly. Six species of trombiculid mites comprising three genera were isolated from soil samples collected from August to October in both 2001 and 2002 at a study site where a man was bitten by *N. nagayoi*. The dominant species was *L. intermedium* (72.4%) followed by *L. pallidum* (8.3%) and *N. nagayoi* (8.1%). *N. nagayoi* was found only in August and September. We did not detect the pathogen *Orientia tsutsugamushi* in any of the unfed larvae, including *N. nagayoi*, collected from the soil samples.

INTRODUCTION

Scrub typhus or tsutsugamushi disease presents following infection by the etiologic agent *Orientia tsutsugamushi* and is transmitted via the bite of unfed trombiculid mite larva. Thus, the vector species and its epidemiological role as a transmitter are determined by studying the infection rate of *O. tsutsugamushi* in suspected mites as well as the behavior of the mites toward humans (Kawamura *et al*, 1995). Historically, in Japan only the three species *Leptotrombidium akamushi*, *L. pallidum* , and *L. scutellare* fulfilled the above conditions and thus were considered to be the main vectors of the disease. However, by 1995 a mouse passage method had been used to confirm that *O. tsutsugamushi* is harbored in 16 species in Japan representing six genera (Takahashi *et al*, 1995; Urakami *et al*, 2000). Therefore, other species besides the three mentioned above may also act as transmitters of *O. tsutsugamushi*, albeit at relatively low infection rates. Indeed, recent studies implicate *L. intermedium* and *L. fuji* as possible vectors (Tamura *et al*, 2000; Urakami *et al*, 2000).

Trombiculid mites have also been a known cause of skin irritation for a long time and have a worldwide distribution (Alexander, 1984). Forty-six species belonging to 13 genera that bite humans have been reported in countries other than Japan (Takahashi *et al*, 1995). In Japan, only 11 of the more than 100 species of trombiculid mites were previously known to bite humans (Asanuma, 1958; Kawamura *et al*, 1995; Suzuki, 1996; Misumi *et al*, 2000). Given that the isolation of new species of vector mites depends in part on demonstrating that their larvae bite humans, a more complete list of those species that exhibit this behavior would be useful. We therefore examined the species composition of trombiculid mites recovered from soil samples collected from late summer to early autumn (August to October) from a study site where a man was bitten by

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N. nagayoi. We showed, for the first time in the world, that the trombiculid mite *N. nagayoi* also causes human cutaneous reactions, thus bringing the total number of such species to 12 in Japan (Table 1). The presence of *O. tsutsugamushi* in these larvae was also examined.

MATERIALS AND METHODS

Study area and soil sampling

The study area was at Togoshinai (40º44′N, 140º19′E, 300 m above sea level) located at the northeastern foot of Mt Iwaki in Aomori Prefecture, Japan (Fig 1), and was planted with ~7,000 *Rhus verniciflua* Stokes trees with low herbaceous plants growing beneath. To examine the number of unfed larval trombiculid mites inhabiting the soil surface, a total of 75 kg of soil samples including humus was collected from sites where the man in the case report was most likely attacked by *N. nagayoi*. We collected the samples on six different occasions during the summer and autumn of 2001 and 2002.

Laboratory procedures

After being transported to the laboratory, soil samples were placed in Tullgren funnels. Unfed larvae that fell onto the surface of water in 9-cm Petri dishes were collected every day for 3 days. These larvae were killed by freezing at -20ºC. Detection of *O. tsutsugamushi* from individual unfed larvae collected from soil samples was performed by a direct immunofluorescent technique described in our previous report (Urakami *et al*, 2000).

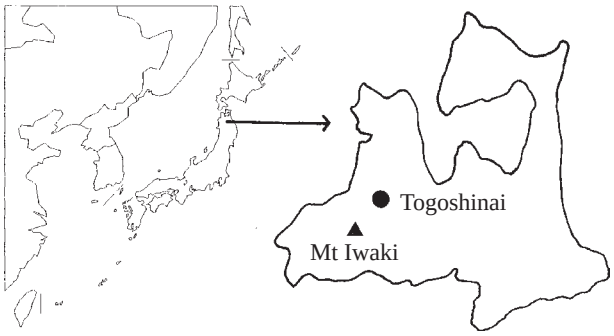

Fig 1–Study site: Togoshinai, Aomori Prefecture, where a man was bitten by *Neotrombicula nagayoi.*

RESULTS

Case report

A 50-year-old male specialist was collecting lacquer from *R. verniciflua* from May to October at Togoshinai at the foot of Mt Iwaki. While resting during midday on 25 August 1999, he felt a slight pruritus with no pain at several sites on his left forearm. He found two orange-colored

Table 1
Larval trombiculid mites that bite humans in Japan.

Species	Locality
Leptotrombidium akamushi	
	Niigata Prefecture (Kawamura, 1925)
	Yamagata Prefecture (Nagayo *et al*, 1920)
	Akita Prefecture (Kitaoka *et al*, 1974)
	Exp (Ito and Obata, 1961; Takahashi *et al*, 1991)
L. scutellare	
	Hachijo Islands (Asanuma *et al*, 1958)
	Nagasaki Prefecture (Toriyama *et al*, 1988)
	Shizuoka Prefecture (Takahashi *et al*, 2000)
	Exp (Kitaoka *et al*, 1953; Toriyama *et al*, 1988; Takahashi *et al*, 2000; Misumi *et al*, 2003)
L. pallidum	
	Niigata Prefecture (Kawamura, 1925)
	Exp (Kitaoka *et al*, 1953; Misumi *et al*, 2003)
L. pallidum burnsi	
	Hachijo Islands (Suzuki *et al*, 1958)
L. palpale	
	Niigata Prefecture (Kawamura, 1925)
Eutrombicula wichmanni	
	Hachijo-kojima Islands (Sasa *et al*, 1958)
	Kagoshima Prefecture (Kitahara, 1952)
Neotrombicula nagayoi	
	Aomori Prefecture (this study)
Shoengastia hanmyaensis	
	Hanmya Island (Suzuki, 1976)
L. fuji	
	Exp (Asanuma, 1959)
L. intermedium	
	Exp (Misumi *et al*, 2000)
L. kitasatoi	
	Exp (Suzuki, 1996)
Gahrliepia saduski	
	Exp (Takahashi *et al*, unpublished data)

Exp=Experimental infestation.

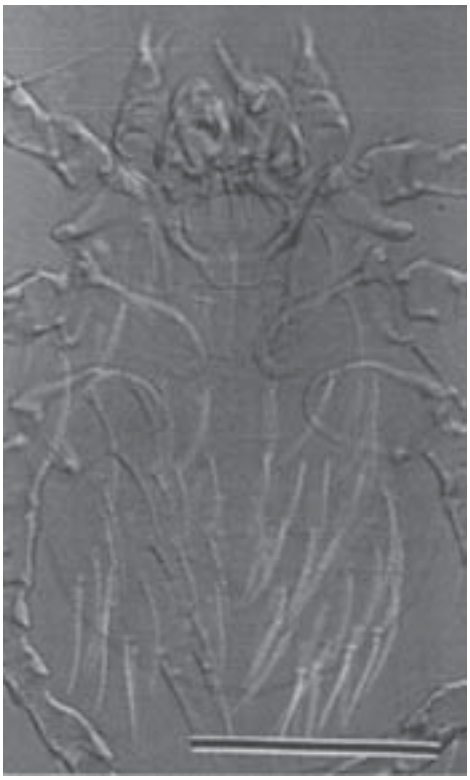

Fig 2–Unengorged larva of *N. nagayoi* extracted by a knife from the subject's left forearm. Scale bar, 100 μm.

mites biting the skin. He forcibly removed the mites and placed them on adhesive tape. The pruritus continued for ~10 days and an erythematous halo developed around the bites. The mites were sent to our laboratory and were identified as *N. nagayoi*. Although he used insect repellent to prevent further attacks, the man was subsequently bitten several times by these mites until the beginning of September 1999.

On 20 August 2000, the man was working at the same site and felt a slight pruritus on his left wrist and forearm. He used a magnifying lens to inspect his skin and found two orange-colored larvae (surrounded by erythematous halos) biting his wrist. He observed two additional larvae as minute orange points in the center of vesicles that were also biting his left forearm. He removed these four mites using a knife. He also found and collected two unfed larvae that had climbed up his right ankle. These six mites were preserved in 70% ethyl alcohol and subsequently mounted on microscope slides. All the mites were identified as *N. nagayoi* (Fig 2).

The man reported that between early August and September 2000 he had been bitten by these larvae in the area surrounding the navel as well as on the back, thigh, ankle, forearm, upper arm, and armpit. He reported that all bites were associated with severe pruritus without pain that continued for 6 to 10 days.

On 29 August 2001 during the third summer season, the mites again bit this man on his upper arm, thigh, and abdomen, resulting in more severe pruritus with pain than he had reported during the previous 2 years. He collected two larvae from his upper arm, and we subsequently identified these larvae as *N. nagayoi*.

Trombiculid mites recovered from soil samples

The mites identified in these samples are shown in Table 2. During this soil sample survey we collected 468 trombiculid mites comprising 6 species representing 3 genera. The most commonly identified species of unfed larvae was *L. intermedium* (72.4%), followed by *L. pallidum* (8.3 %) and *N. nagayoi* (8.1%). The other three species collected, *L. palpale*, *N. japonica* and *Gahrliepia saduski* are listed in numerical order in Table 2. *N. nagayoi* was identified only in those soil samples collected in August and September.

Detection of *O. tsutsugamushi* from unfed larvae

The presence of *O. tsutsugamushi* was evaluated in 367 unfed larvae collected from soil samples (larvae consisted of five species, *L. intermedium*, 290; *L. pallidum*, 33; *L. palpale*, 7; *N. nagayoi*, 34; *N. japonica*, 3. Although these five mite species were known to carry these organisms in other areas in Japan and in other countries (Gopachenko *et al*, 1976a, b; Somov *et al*, 1976a, b; Takahashi *et al*, 1995; Urakami *et al*, 2000), no larvae tested positive for these organisms in the present study.

DISCUSSION

Neotrombicula nagayoi is one of the mite species that forms larval clusters on the ground

Table 2
Trombiculid mites recovered from soil samples in the study site Togoshinai, Aomori Prefecture, Japan in 2001 and 2002.

Date	2001				2002		Total	
	26 Aug	10 Sept	11 Oct	19 Oct	16 Aug	15 Sept		
Soil samples (kg)	5	10	20	10	15	15	75	(%)
L. intermedium	3	37	94	6	81	118	339	(72.4)
L. pallidum		1	21	1	1	15	39	(8.3)
L. palpale			8			1	9	(1.9)
N. nagayoi	1	7				30	38	(8.1)
N. japonica			3	1			4	(0.9)
G. saduski			1				1	(0.2)
Nymph			1	2			3	(0.6)
Adult	4	6	12		8	5	35	(7.5)
Total	8	51	140	10	90	169	468	

L, Leptotrombidium; N, Neotrombicula; G, Gahrliepia.
All trombiculid mites except for nymphs and adults were unfed larvae.

surface and/or the tips of plants and leaves. In the case report from the present study, a man was bitten many times on various parts of his body by unfed *N. nagayoi* larvae. Hubert (1971) observed that *N. nagayoi* cluster on the lower parts of vegetation at the Camp Fuji area in Japan. The clusters occurred at an average height of 3.6 cm above the ground, with a range of 1 to 10 cm. In comparison, *L. intermedium* and *L. pallidum* form clusters in small holes and/or crevices on the ground surface rather than on vegetation (Takahashi *et al*, 1991; Misumi *et al*, 2003). Clustering at a higher level such as the tips of slender branches or the extreme points of grass offers an apparent advantage in that many unfed larvae can simultaneously board passing animal hosts, including humans. Moreover, it is relevant that *N. nagayoi* may have a greater tendency to bite humans than *L. intermedium* and *L. pallidum* larvae.

As shown in Table 1, three of the 12 species proven to bite humans (*L. akamushi, L. palpale,* and *Eutrombicula wichmanni*) in Japan induce reactions that tend to be severe and painful. In those individuals bitten by the other nine species, however, a mild pruritus is usually felt, although they often do not realize that a mite bite is the source of their discomfort (Suzuki, 1976; Misumi *et al*, 2000). In most cases, however, the bite of larval trombiculid mites such as *L. intermedium*, *L. pallidum* and *L. scutellare* does not lead to the formation of bullae (Misumi *et al*, 2000; 2003; Takahashi *et al*, 2000). In the present study, the symptoms of the male subject's cutaneous reactions gradually increased year by year after being bitten repeatedly by *N. nagayoi*.

The development of immunity against trombiculid larvae bites is considered to be similar to that developed against other arthropods (Alexander, 1984). Permanent or long-term human residents in an infested area increase their immunity as a result of continued bites and develop a high degree of tolerance to the antigenic substances injected by the larvae as they bite the victim. However, the occurrence of unusual outbreaks of urticaria, increasingly severe pruritus or bulla formation, are indications of hypersensitivity to such antigenic substances. The man in this study had probably developed a sensitivity rather than increased immunity to the bites.

Neotrombicula nagayoi is distributed primarily throughout northern Japan, China, and Russia (Gopachenko *et al*, 1976a,b; Somov *et al*, 1976a,b; Takada, 1976, 1990; Wen, 1984). This mite appears seasonally from August to November and is relatively abundant in the autumn in the Tohoku dis-

trict of Japan (Takada, 1976). The data collected for *N. nagayoi* in the present study are in agreement with those of the previous studies mentioned above.

The pathogen for scrub typhus, *O. tsutsugamushi*, was not detected in unfed larvae including *N. nagayoi* collected in this study. This pathogen was, however, isolated from engorged larvae of *N. nagayoi* recovered from field rodents in Far Eastern Russia (Gopachenko *et al*, 1976a,b; Somov *et al*, 1976a,b). To establish that a particular vector transmits scrub typhus it is necessary to not only demonstrate that the mite species bites humans but also that unfed larvae harbor *O. tsutsugamushi*. At present, *N. nagayoi* mites cannot be considered as proven vectors for scrub typhus in Japan. Further studies, however, may show that unfed larvae collected from other sites do, in fact, harbor *O. tsutsugamushi*. Our current study establishes the groundwork for rapid identification of scrub typhus vectors using the above criteria.

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