ON THE NYMPH AND PROSOPON OF THE TSUTSUGAMUSHI, LEPTOTROMBIDIUM AKAMUSHI, N. SP. (TROMBIDIUM AKAMUSHI BRUMPT), CARRIER OF THE TSUTSUGAMUSHI DISEASE.

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Plates 24 to 27.

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INTRODUCTION.

Tsutsugamushi or kedani disease is an acute exanthematous infectious disease, which at present occurs only in the northern coast districts of Japan; *i.e.*, in Niigata ken, Akita ken, and Yamagata ken. Recently it has also been found to exist in Formosa (1). The mortality of the disease varies, according to the district and year, between 20 and 50 per cent. It closely resembles Rocky Mountain spotted fever, but, as Ashburn and Craig (2) have pointed out, it possesses some distinct features. Among previous investigators of the disease may be mentioned Baelz, Kawakami, Tanaka, Kitasato, Ogata, Asakawa, Kitajima, Miyajima, Hayashi, Kawamura, Arima, Sakai, and Hattori, who have studied the etiological, pathological, and clinical features. The specific cause of the disease has not yet been established with certainty. Last year we undertook to investigate the disease, and especially the question as to its cause and means of transmission. As regards the specific germ we expressed the opinion that certain piroplasma-like forms discovered by us, which occur in the spleen, lymphatic glands, and in the blood, but not in the red corpuscles, and which may belong to the Sporozoa, may be the cause (3). We shall not describe them here in detail, but shall confine our report to the carrier of the disease, a species of Trombidium.

That tsutsugamushi disease is transmitted to man by a diminutive mite, is now established. Baelz (4), to whom we owe the first scientific description of the disease, was at first not inclined to admit this fact, but it was undeniably proven by later investigators through clinical observation and experiments on animals. This mite is called in Japan tsutsugamushi (disease mite), akamushi (red mite), kedani (hairy mite), or shimamushi (island mite), and is similar to the European harvest-bug, *Leptus autumnalis* (Baelz). Tanaka (5) was the first to describe it in detail. The specific determination was first undertaken in 1910 by Brumpt (6), who considered it to be a larva of a new trombidium, which he called *Trombidium akamushi*, apparently, however, without finding its nymph and prosopon. Recently Kawamura and Komagata (7) have published a detailed description of the external and internal structures of this mite. The studies mentioned above refer only to the larva of a hitherto unknown trombidium, *Trombidium akamushi* Brumpt, which attacks warm-blooded animals as well as man. The nymph and the prosopon of the tsutsugamushi, however, do not bite warm-blooded animals and have not yet been detected with certainty. Several authors claim to have discovered or bred the nymph or the prosopon, but their reports are not convincing. Some trombidium larvæ are well known in Europe and America under the names *Leptus autumnalis*, *Leptus irritans*, *Leptus americanus*, harvest-bug, redbug, chigoe, etc. They also bite human beings and are more or less closely related to the tsutsugamushi; the other larvæ, however, never cause so serious a disease as the latter. That the investigation of this mite has so far been neglected is chiefly due to the danger of the bite which causes the disease, and to which the investigators are exposed while working in the infected region.

Since beginning the present investigation, from November, 1915, until August, 1916, we have frequently been in the infected districts from 2 days to 1 week every month. In summer, when there is danger of being bitten by the mite, we dressed in such a way as not to expose any part of the body except the eyes; and we rubbed the body with some mite-killing agent, such as phenol, sulphur ointment, or insect powder. At other seasons, when there is usually no danger of being bitten, we could work without special precautions. Of the endemic regions in Japan we selected as a place for research the environs of Yachi on the banks of the Mogami river in Yamagata ken, which for the last 3 years has been known as an endemic district. We visited the district near Yuzawa in Akita ken once only. In the suburbs of Tokyo, where the tsutsugamushi is not found, and consequently the disease does not occur, we have on several occasions collected various trombidia. After investigations extending

over 1 year, we are now in a position to report on the nymph and the prosopon of the tsutsugamushi. We were often misled by the circumstance that in the endemic district various kinds of trombidia are found, which in contrast to the prosopon of the tsutsugamushi are more striking in size and color, and therefore more easily found than the prosopon of the tsutsugamushi. At first we mistook a particular species of trombidium for the parent of the tsutsugamushi, as other authors have also done both before and after us. Subsequently we found that there occur a number of species of trombidium in the endemic region and we have been able to isolate with certainty at least five species, which we have called provisionally Trombidia A, B, C, D, and E (8).

Trombidia A, B, C, and D lay their eggs in culture soil, and we were able to raise larvæ from the eggs. With Trombidium B we succeeded in raising nymphs from the larvæ. We also found the nymphs of Trombidia A, B, and C in nature. We shall not go into detail about the various species of trombidium, but merely point out that Trombidium B may be regarded as a variation of *Trombidium fuliginosum* Herm, and that Trombidium D cannot be distinguished, at least morphologically, from the European *Trombidium holosericeum* Linné. Trombidia A, C, and E are perhaps new species.

The larva of Trombidium B somewhat resembles the tsutsugamushi, and at first we believed them to be identical. In raising the eggs of Trombidium D we watched their development closely, because we thought that we might be able to raise tsutsugamushi from them, as *Trombidium holosericeum* has generally been considered to be the parent of *Leptus autumnalis* (Mégnin (9)), and the latter is so closely related to the tsutsugamushi that it may only be a variation of it, and as *Trombidium holosericeum* corresponds to Trombidium D. But since the larvæ obtained differed in essentials from tsutsugamushi and from *Leptus autumnalis*, we were forced to the conclusion, that *Trombidium holosericeum* Linné, is not the parent of *Leptus autumnalis*, as some authors have observed.[1] We believe that we have found the real parent of the tsutsugamushi, and we wish to report

[1] See Nagayo, M., Miyagawa, Y., Mitamura, T., and Imamura, A., Is *Trombidium holosericeum* the Parent of *Leptus autumnalis*, *J. Exp. Med.*, 1917, xxv, 273.

here on the nymph and the prosopon of the tsutsugamushi, which have hitherto remained unknown.

The Nymph of the Tsutsugamushi.

On April 30 and May 2, and 3, 1916, we received from Okiage and Nakagawara numbers of tsutsugamushi-carrying field mice. The lobes of their ears, which were thickly covered with the mites, were cut off and put into a glass dish. The mites then gradually loosened their hold and left the host, the fully fed ones sooner than the others. The mites were put in glass bottles, test-tubes, and glass dishes, which had been provided with moistened soil from the endemic districts, thoroughly sterilized by heat. On June 8, about 40 days later, three nymphs were found in one bottle, and later more (twenty-one in all) in the other vessels. Hayashi (10) and Kitajima and Miyajima (11) claim to have bred nymphs artificially before, but they do not describe the nymphs adequately, so that we cannot compare ours with theirs. We experienced no great difficulty in the culture of the nymphs. It is necessary to protect the soil from drying and rotting during the period of the experiment. The nymphs show great motility and like to hide in the ground. The second cultivation experiment, which we undertook with the larvæ taken from field mice caught on July 19, was likewise successful, and took a much shorter time than the first. After 10 days, on July 29, we found small nymphs crawling about, upon and in the soil of the vessels. The total number of nymphs produced amounted this time to over 80. They were a little smaller than those of the former breed, so it may be presumed that in the first experiment the nymphs were already some days old when they were discovered.

On July 30 we received two field mice from Nakagawara and for the third time we used the numerous attached tsutsugamushi for breeding the nymphs. For the purpose of studying the evolution of the pupa we distributed the well fed larvæ in eight breeding vessels, each of which contained 20 to 30 larvæ. After 3 full days, *i.e.*, on August 2, the first vessel was examined, and the others one by one on each succeeding day. This time the nymphs came out after 8 days, on August 7. The metamorphosis of the larva into the nymph

proceeds, though subject to slight variation, in general in the same manner as that described by Henking (12) for *Trombidium fuliginosum*. On the 4th day we already found the nymphophane. The oval immovable pupa is in every case distinguished by the formation of the so called *Apoderma* of Henking (the *Zwischenhaut* of Claparède (13)), lying under the larval cuticle. This is nearly transparent and supplied with single granule-holding round cells, which may, however, occasionally form groups (*Haemamoeben* of Claparède). Below this skin is the thin nymphal cuticle. The new extremities are already budding out and are likewise enveloped by the apoderm. There are now four pairs, the fourth pair being a new formation, as Henking has already described in *Trombidium fuliginosum*. The original three pairs of legs and the mouth apparatus have already undergone complete histolysis, so that nothing is visible under the original larval skin. The legs break off easily and are always outstretched, they never take the bent position, which is often found in dead larvæ. The original mouth apparatus is pushed somewhat forward and is easily detached from the body of the nymphophane. In the course of the following days the newly formed legs, of which the first pair is the best developed, grow longer and show a distinct arrangement of the parts and claws. They are directed obliquely backwards and towards the middle, so that the distal ends of each pair touch in the median line.

We have less to say of the development of the mouth apparatus. On the 4th day a small hemispherical protuberance is seen under the original mouth part of the larva, completely separated from the latter by the apoderm. Within this prominence one sees the anlage of the mouth parts; on the following days one can gradually distinguish the palpi, directed ventrally and backwards, and the mandibles between them. On the dorsal side of the head a peculiar spur-like structure is seen, with its point directed anteriorly. It projects from the chitinous skin of the nymph and reaches through the apoderm almost to the original larval skin. Henking does not mention this structure in *Trombidium fuliginosum;* it is probably an organ of the nymph to enable it to come out of the larval cuticle. In an 8 day old nymphophane we had excellent proof of this; we saw how the chitinous cuticle of the larva was pierced and torn by

this spur. The apoderm meanwhile increases in thickness and becomes provided with numerous acuminate excrescences. Later it grows thinner again, often becomes folded, and shows irregularly stratified lamellar structures. Through this apoderm the underlying newly formed hairs of the nymph are seen. The round cells, fairly numerous at one time, gradually disappear again. Shortly before the nymph emerges, the apoderm undergoes granular degeneration. Thus the evolution of the nymph is completed within a relatively short time, so that on the 9th day the majority of the new nymphs are found in or upon the soil of the receptacle.

Morphology of the Nymph.

Size and Form.—The nymph is a small, rather long, oval mite with eight legs, and shows between the cephalothoracic region and the abdomen a distinct constriction, especially conspicuous during life. The thorax and abdomen are correspondingly rounded at the sides, and can easily be distinguished. The length of the body from the points of the mandibles is 0.342 to 0.644 mm.; the maximum width of the cephalothoracic region is 0.168 to 0.280 mm.; and that of the abdomen 0.198 to 0.324 mm.

TABLE I.

Measurements of the Legs of a Nymph 0.612 Mm. in Length.

	Total length.	Joint.					
		1	2	3	4	5	6
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1st pair.							
Length	0.432	0.028	0.060	0.060	0.072	0.088	0.124
Breadth		0.036	0.036	0.046	0.052	0.060	0.060
2nd pair.							
Length	0.236	0.023	0.028	0.028	0.040	0.040	0.068
Breadth		0.036	0.040	0.036	0.034	0.028	0.024
3rd pair.							
Length	0.208	0.024	0.024	0.024	0.028	0.044	0.064
Breadth		0.040	0.028	0.032	0.028	0.024	0.020
4th pair.							
Length	0.284	0.040	0.040	0.028	0.044	0.052	0.080
Breadth		0.036	0.032	0.032	0.028	0.028	0.024

Color.—The body is grayish white or pale yellow, while the legs are colorless. The contents of the stomach shining through the body give it the yellow color. The hairs and epidermis are colorless.

Legs.—There are four pairs of legs. The first pair is the longest, the fourth pair coming next, then the second and third (Table I).

The anterior pair is by far the longest; the segments, in contrast to the other legs, become longer and broader towards the distal end. Each tarsus is provided with two claws but has no clinging hairs. The claws of the anterior pair are much shorter (length 0.016 mm.) and less curved than those of the other legs (0.022 mm.).

Epimeres.—The epimeres of the first and second pairs of legs of the same side lie close together, as do those of the third and fourth pairs, though nowhere grown together, so that they form on each side an anterior and a posterior group. The surface of the epimeres is finely punctated. The first pair of epimeres extends distally for about one-third of their length over the edge of the body.

Measurements of the Epimeres.

	Leg.			
	1	2	3	4
	mm.	mm.	mm.	mm.
Length	0.080	0.060	0.068	0.076
Width	0.044	0.048	0.052	0.048

	mm.
Distance between the anterior and posterior groups	0.048
Transverse distance between the pairs of the anterior groups	0.020
" " " " " " " posterior "	0.060

Tracheal and Stigmatal Plate.—We have not yet found with certainty the tracheal openings which are distinctly visible in the larvæ on the first pair of the epimeres. At least the scaly chitinous structure surrounding the tracheal openings, Henking's *Stigmenschutzapparate*, which is distinguished in the nymphs and prosopa of other trombidia, is not distinctly developed in ours.

Genital Opening and Anus.—The genital opening is oval and is situated in the median line of the ventral side of the abdomen, behind the fourth epimeres. The anus opens further back.

	Genital opening.	Anus.
	mm.	*mm.*
Length (sagittal diameter)	0.072	0.056
Width (cross diameter)	0.048	0.040

The genital opening is surrounded on either side by a raised portion, provided with two flat oval plates, sucking discs.

Mouth Apparatus.—The palpus consists of four joints besides the basal part. The second is the largest in size. The terminal joint is provided with a claw (length 0.024 mm.) and a longer appendage, the thumb (length 0.036 mm.). This appendage is not club-shaped, as is usual with trombidia, but tapers towards the end and is covered with numerous pinnate hairs. If we regard this appendage as an independent joint of the palpus, as is done by Banks (14), Fiebiger, and others, we may count five joints in the latter.

Size of the Palpi.

	Joint.			
	1	2	3	4
	mm.	*mm.*	*mm.*	*mm.*
Length	0.016	0.048	0.036	0.034
Width (greatest)	0.020	0.044	0.032	0.020

Mandibles.—The mandible, which averages 0.112 mm. in length has at the front a sharp, dorsally curved hook (length 0.052 mm., width 0.020 mm.), with fine teeth on the concave edge. Both mandibles are imbedded in a boat-shaped area, which gradually tapers towards the front and is pilose. The mandibles are covered at the posterior part by a corrugated epithelium, an elongation of the dorsum of the body, the epistome. A little in front of this epistome there is one hair, feathered and pointed, 0.0334 mm. long, in the median line between both mandibles.

Scutum.—The scutum consists of two symmetrical circular parts (the distance between them 0.020 mm.), each bearing a long hair (length 0.100 mm.), and a middle part connecting the two, which extends behind into a short process and forward into a hairless chitinous crista reaching to the end of the epistome.

Eyes.—The eyes are not easily visible. They are sessile and situated close to the pseudostigma of the scutum. The eye contains

fine reddish granules, but is on the whole rudimentary, compared with that of the larva, and is not provided with clearly developed lenses.

Hairs.—The paired tactile hairs of the scutum have already been mentioned. The entire body of the nymph is thickly covered with hairs. The most striking are those on the back of the abdominal segment, which are longer than the others (0.056 to 0.080 mm.), and have a rather thick shaft and club-shaped end. The surface of the shaft is covered with innumerable small pinnate hairs. The clubbed shape of the end varies in different individuals, and probably depends on the nourishment and state of development of the nymph. The length of the hair, too, shows individual variations. These hairs seem to be peculiar to the nymph of the tsutsugamushi, and have so far been found besides only in *Trombidium holosericeum* among our trombidium species. They differ from the latter in that they are colorless, while the club-shaped hairs of *Trombidium holosericeum* are red. They arise from distinctly developed hair follicles. In other parts of the body the hairs are generally shorter (0.028 to 0.050 mm.) On the anterior part of the cephalothoracic region, especially before the scutum, there is little hair. The hairs of the legs and mouth apparatus are not clubbed but simply pinnate and pointed, and on the whole similar to the pinnate hairs of *Trombidium fuliginosum* and our Trombidia B and C. The hairs are especially numerous on the first pair of legs and on the appendage of the palpus.

The Prosopon of the Tsutsugamushi.

Encouraged by the result of breeding the nymphs, we tried to find them in the endemic district, and succeeded after great difficulty. On July 13 we found 3 specimens in Horiguchi, 8 and 7 respectively on July 17 and 18 in Okiage, 7 on July 19 in Nakagawara, 1 on July 31 in Horiguchi, 15 on August 1 in Nakagawara, 1 on August 9, and 23 on August 14, making a total of 65 specimens. It requires great patience to search in the oppressive heat of summer, with the entire body carefully covered against the larval bite, for this tiny pale colored insect which can be distinguished from a grain of sand only by its movement. We often came home without results. One

day, when five persons took part in the hunt, only one specimen was found. The tsutsugamushi are usually found in the ground, and when brought to light by the turning of the soil, they try to hide again in the earth. Their color is sometimes grayish white without any yellowish red shade, but usually pale yellow or orange. In the latter case the hairs remain colorless. The body of the animals is always considerably larger than that of artificially bred nymphs as is shown in Table II.

TABLE II.

No.	Length.	Greatest width.		Color.
		Thorax.	Abdomen.	
	mm.	*mm.*	*mm.*	
1	1.166	0.564	0.720	Pale yellow.
2	0.738	0.415	0.476	Gray.
3	0.918	0.424	0.600	"
4	0.922	—	—	Orange-yellow.
5	About 1.000	—	—	Pale yellow.
6	" 1.000	—	—	Orange-yellow.
7	0.886	0.416	—	Gray.
8	1.152	0.612	—	"
9	0.810	0.450	0.486	Pale yellow.
10	1.080	0.540	0.700	" orange.
11	1.250	0.630	0.900	Orange.
12	1.260	0.620	0.810	"
13	0.950	0.584	0.680	Grayish white.
14	1.116	0.504	0.648	Pale orange.
15	0.990	0.396	0.414	Stained.
16	1.440	0.720	0.900	Orange.
17	1.260	0.594	0.756	"
18	1.170	0.504	0.648	"
19	1.440	0.840	1.080	Pale orange.
20	1.260	0.680	0.936	Orange.

When we first discovered these mites we were of the opinion that we had before us the free living nymphs of the tsutsugamushi, for we found with the exception of the difference in size and a deeper coloring in many specimens no essential difference in outward appearance from the artificially reared ones. But when we made a minute comparison, we found a difference in the number of sucking discs

of the genital opening. While the nymphs, as already described, always have two pairs, we counted in this case three pairs of discs. Banks (14) has already pointed out that in certain mites, especially in Hydrachnidæ which resemble Trombidia, the shape and number of the sucking discs are important characteristics in distinguishing the species. We found in Trombidia A and B always three pairs, in the nymph as well as in the prosopon, while Trombidium C has only two in the nymph and three in the mature animal.

It therefore seems that the animals caught by us were not nymphs but mature animals. We have hesitated to pronounce them as such until we succeeded in finding eggs in their body. In animals caught on July 31 and August 1 we found eggs in various stages, which could be distinctly seen by pressing out the contents of the stomach under a cover-glass. The mature eggs are round, and have a relatively thin shell and an almost colorless yolk; their diameter is about 0.162 mm. Besides these a few immature, mostly smaller eggs can be seen through the skin. But we did not find any prosopa rich in eggs. It is probable that they deposit their eggs only in small quantities; at any rate, it seems that they never deposit a single heap formed of hundreds of eggs, as is the case with other trombidia.

Measurements of the various parts of a prosopon 1.166 mm. long are given in Table III.

TABLE III.

	mm.
Width of thorax	0.504
" " abdomen	0.720
" " constricted part	0.468
Distance of the third pair of coxæ (epimeres)	0.120
Length of mandibles	0.180
Width " "	0.068
Length " mandible hook	0.068
Total length of palpi with basal joint	0.344

TABLE III—*Continued.*

Palpi.

		mm.
Basal joint.	Length	0.120
	Width	0.042
Joint 1.	Length	0.024
	Width	0.040
" 2.	Length	0.084
	Width	0.044
" 3.	Length	0.056
	Width	0.040
" 4.	Length	0.060
	Width	0.024
Claw		0.020

Leg.

		1	2	3	4
		mm.	mm.	mm.	mm.
Coxa.	Length	0.120	0.080	0.112	0.112
	Width	0.056	0.048	0.090	0.090
Joint 1.	Length	0.064	0.048	0.044	0.052
	Width	0.048	0.044	0.040	0.044
" 2.	Length	0.056	0.048	0.048	0.060
	Width	0.048	0.040	0.038	0.042
" 3.	Length	0.088	0.048	0.044	0.060
	Width	0.060	0.040	0.038	0.044
" 4.	Length	0.108	0.060	0.056	0.068
	Width	0.060	0.044	0.038	0.040
" 5.	Length	0.112	0.064	0.068	0.088
	Width	0.064	0.044	0.038	0.040
" 6.	Length	0.200	0.108	0.104	0.132
	Width	0.072	0.072	0.034	0.036
Claw		0.028	0.036	0.033	0.032
Total length of legs without epimeres		0.628	0.378	0.364	0.460

		mm.
Width of dorsal shield		0.072
Greatest sagittal diameter of dorsal shield		0.038
Length of tactile hair		0.120
Genital parts.	Length	0.113
	Width	0.080
Genital opening.	Length	0.060
	Width	0.036
Sucking discs.	Length	0.028
	Width	0.036
Anus.	Length	0.048
	Width	0.056
Distance between anus and genital opening		0.080
Length of dorsal hairs		0.056–0.068
" " hairs of legs		0.032–0.042

Morphologically the prosopa of the tsutsugamushi, differ greatly from other trombidia. Their chief characteristics are their small size, pale color of body, indistinct eyes and stigma. They are also characterized by the distinct constriction between the cephalothoracic region and the abdomen and the peculiarity of their hairs.

Sections have so far been made of six nymphs and twelve mature animals. Except for the genital gland both forms show in their inner morphology practically no differences. They have, compared with other species, a comparatively soft chitinous coat, so that for making serial sections a short immersion in alcohol or ether is sufficient. Both forms have a large brain and a lobed stomach. The intestine, esophagus, fat bodies, etc., are like those of the other trombidia. The large paired salivary glands possess several lobes and show three different kinds of cells according to their protoplasm or enclosed granule. One variety has fine granules which stain weakly with eosin, and the second coarse, distinctly eosinophilic granules, while the third consists of almost transparent cells filled with numerous vacuoles. Occasionally all three kinds of cells are found in the same lobe. At least two ducts are visible, which later unite. The Malpighian tubule consists of a pair of tubes. Both ends lie on the ventral side of the salivary gland and genital organ, the middle part being situated near the brain and the stomach.

The tracheal system is extraordinarily rudimentary, as are the eyes.

We were unable to find any tracheal capillaries, while in Trombidia A, B, C, and D numerous tracheas are easily seen in almost all parts of the body.

Regarding the genital gland, we found in the artificially bred nymphs only cell heaps directly behind the brain, between the genitalia and the stomach, which may be regarded as the undifferentiated germ cells. The animals caught in the open, on the contrary, show a well developed gland; *i.e.*, testicles or ovaries. In the female there is an oviduct on either side and a uterus provided with a muscular wall. The ovary contains ova in various stages of development. In three cases we found eggs already enclosed in the shell.

According to the external and internal morphological structure of the prosopa there is no possible doubt that we have before us the mature form of the tsutsugamushi.

We should mention that our nymph of the tsutsugamushi is closely related to that of *Leptus autumnalis*, artificially bred by Brandis (15), while the prosopon of the tsutsugamushi is different from *Trombidium holosericeum;* the latter, however, has, since Mégnin, often been considered the parent of *Leptus autumnalis*.[1]

Cultivation of the Larva of the Tsutsugamushi.

After we had discovered the prosopon of the tsutsugamushi in the open field (16), we tried to breed larvæ (tsutsugamushi) from the mature animal. To this end we distributed many prosopa into a number of small glass vessels and examined each vessel every 2nd day. On August 18 we found for the first time three small, yellowish red insects crawling about on the side of one of the vessels, which after being fixed in Berlese's fluid were found to be tsutsugamushi larvæ. The next day four larvæ were discovered in another vessel. No eggs were found in the soil of the two vessels, one of which on previous examination had been found free of trombidium eggs, while the other had been sterilized by heat. In the investigation of Trombidia A, B, C, and D we had found heaps consisting of hundreds of eggs. In one of the vessels we found the dead bodies of two prosopa. The hatched larvæ are undoubtedly the true tsutsugamushi; *i.e.*, they show no essential difference from tsutsugamushi larvæ taken from field mice and man; but they are distinctly smaller than those that have fed on the lymph of warm-blooded animals; they appear almost round in contrast to the more oval shape of the latter. Moreover, larvæ which have not yet sucked on mammals, have a broader mouth part and a more deeply colored body, which is orange-red in appearance. Otherwise the palpi, legs, epimeres, hairs, stigmata, eyes, and the appearance of the body are alike in both. Though we have not yet found eggs in the soil of the receptacle, it seems certain that the hatched larvæ are the offspring of the prosopa, because the soil had been thoroughly sterilized by heat before the latter were put in. There are two possibilities as to the way in which the larvæ develop; either the prosopa deposit so few eggs that they easily escape the attention of the observer, or no eggs are deposited, the larvæ leaving the body of the parent already formed

(viviparous). At any rate, by the artificial breeding of the larvæ the parent of the tsutsugamushi has been definitely established, and the tsutsugamushi is disinct in this as it is in the nymph and prosopon stages.

SUMMARY.

Nomenclature.

As mentioned above, the prosopon and the nymph of the tsutsugamushi have many characteristics which distinguish it from the other trombidia. These are the pale color and small size of the body, delicacy of the skin, the conspicuous constriction between the cephalothoracic region and the abdomen, rudimentary eyes and tracheal openings, absence of tracheal capillaries, etc. The fact that the thumb or the appendage of the palpus does not start from the side of the fourth joint of the latter but from its end, is another characteristic which does not occur in other trombidia. From the biological point of view two facts should be mentioned; *i.e.*, the adult female does not deposit eggs in heaps, and in contrast to those of other species, the larvæ hatch out all through the year and feed on mammals. From these characteristics running through all the stages of development, we believe that we are justified in claiming a new genus for the tsutsugamushi. We therefore propose to change the scientific name given to this mite by Brumpt in 1910, *Trombidium akamushi*, and to introduce instead, according to the suggestion of Drs. Goto and Watase, the name *Leptotrombidium akamushi*.

Determination of Leptotrombidium akamushi, N. Sp.

Prosopon and Nymph.

1. Pale color.
2. Delicacy of the skin.
3. Distinct constriction between the cephalothoracic region and the abdomen.
4. Rudimentary, sessile eyes.
5. Indistinctness of tracheal openings.
6. Absence of tracheal capillaries.
7. Crista abruptly widened at the end.
8. One palpal claw.

Larva.

1. One scutum.
2. Round spiracles.
3. Legs, six joints, not counting the coxa.
4. Hairs on the coxa: one each on Joints 1, 2, and 3.
5. Hairs on the scutum, including two tactile hairs, seven; one is in the median line.

Specific Determination of Leptotrombidium akamushi, N. Sp. (Trombidium akamushi Brumpt, 1910).

Prosopon.

1. Small size.
2. Three pairs of sucking discs on the external genitalia.
3. Hairs of the body feathered, club-shaped at the end, and colorless.
4. The appendage of the palpus tapers distinctly towards the end.

Nymph.

1. The size of the body is smaller than that of the prosopon.
2. Two pairs of sucking discs on the external genitalia.

There are no other distinct differences in the external morphology between the prosopon and the nymph.

Larva.

1. The color of the body is orange-red, but becomes paler after sucking on mammals.
2. The hairs of the body average 110 to 120 in number.
3. One pair of double eyes.
4. The sucking tube is visible when feeding on mammals (Hayashi).
5. The salivary gland is relatively large (Kawamura and Komagata).
6. The number and arrangement of the hairs on the legs and mouth parts correspond to the description given by Hirst.
7. The larvæ are found all through the year.
8. The larva is the carrier of tsutsugamushi disease in man.

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EXPLANATION OF PLATES.

PLATE 24.

FIG. 1. Tsutsugamushi larva artificially bred. × 143.

FIG. 2. Tsutsugamushi larva from man, fully fed. × 135.

FIG. 3. Tsutsugamushi nymphophane on the 2nd day of breeding. × 79.

FIG. 4. Tsutsugamushi nymphophane on the 8th day of breeding. × 79.

FIG. 5. Tsutsugamushi nymph artificially bred from larva, seen from the ventral side. × 79.

PLATE 25.

FIG. 6. Tsutsugamushi nymphophane on the 7th day of breeding. The original larval skin is pierced by a spur-like structure of the nymphophane (left side upwards). × 220.

FIG. 7. Tsutsugamushi nymph artificially bred from larva. × 200.

PLATE 26.

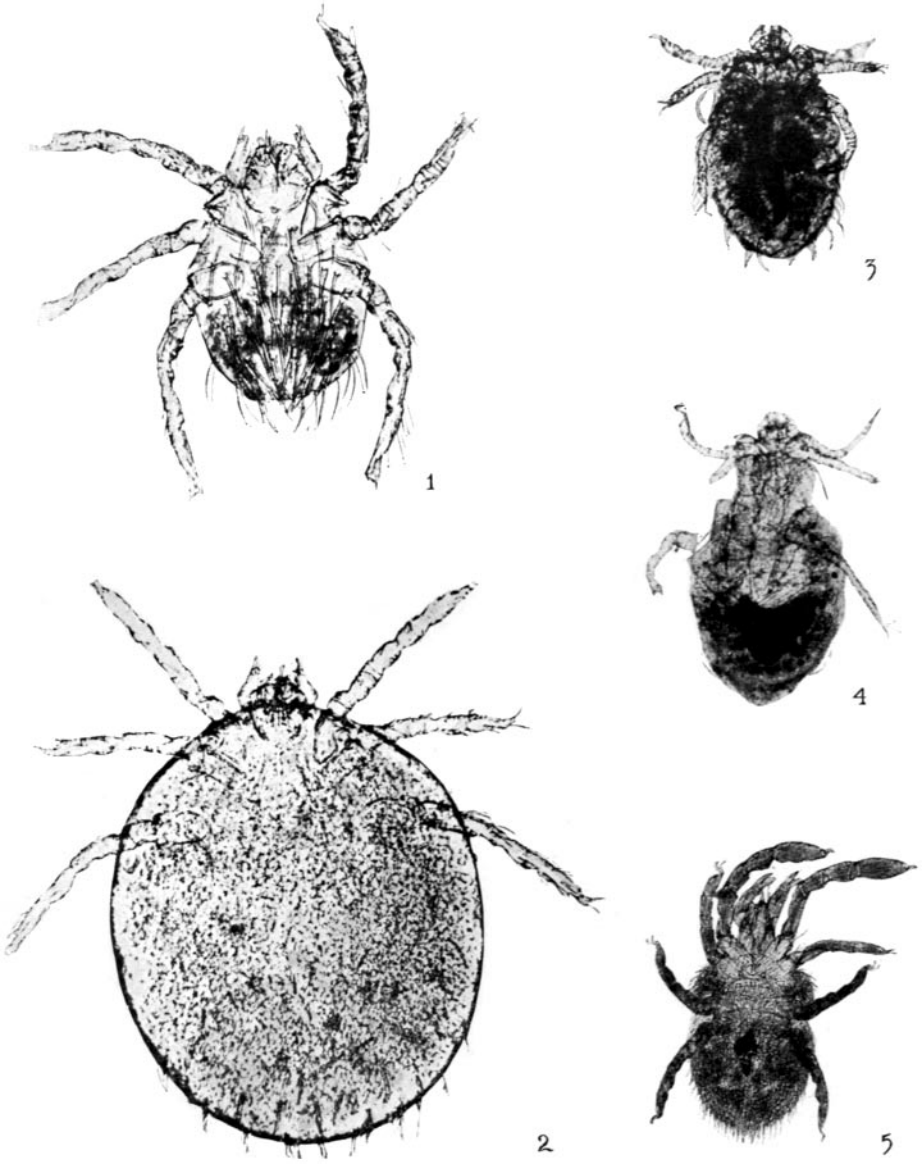
FIG. 8. Tsutsugamushi prosopon (captured specimen). × 81.

FIG. 9. Tsutsugamushi. Sagittal section of a male prosopon. × 97.

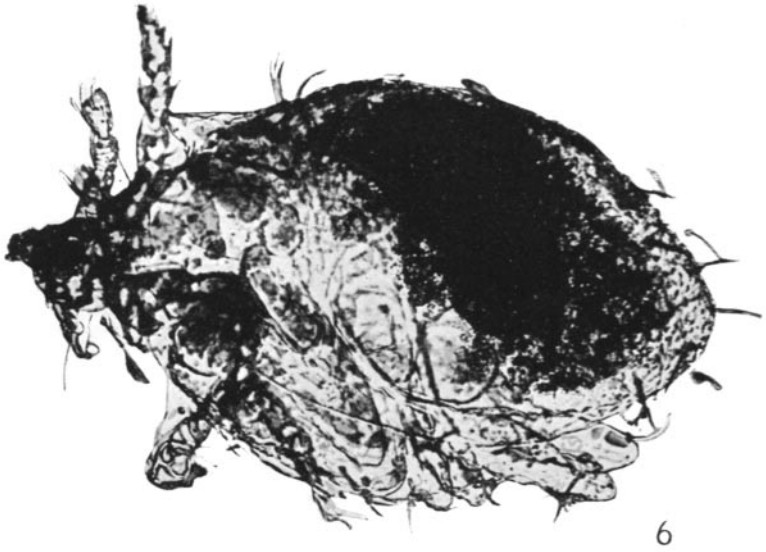
PLATE 27.

FIG. 10. Tsutsugamushi. Sagittal section of a female prosopon. × 100.

FIG. 11. Field work in Okiage, one of the endemic districts in Yamagata ken, July 18, 1916.



(Nagayo, Miyagawa, Mitamura, and Imamura: The Tsutsugamushi.)



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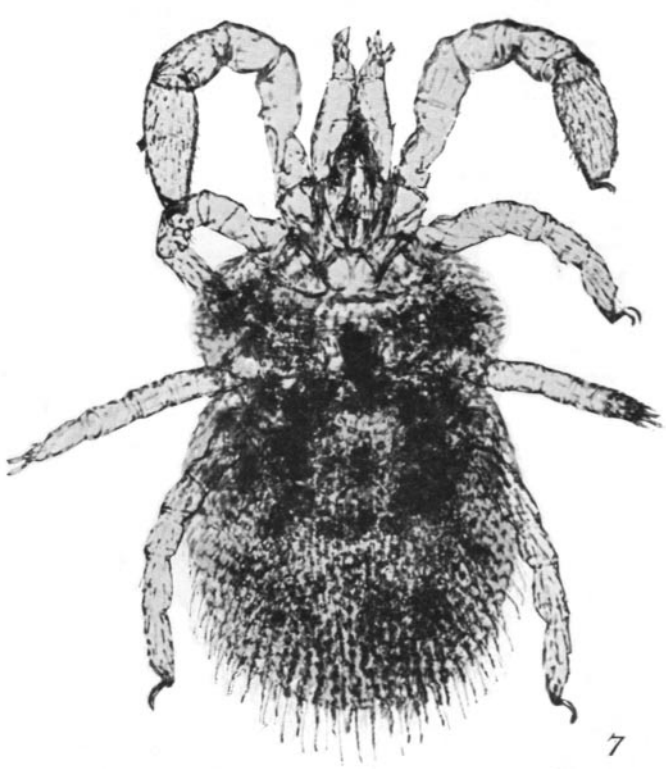

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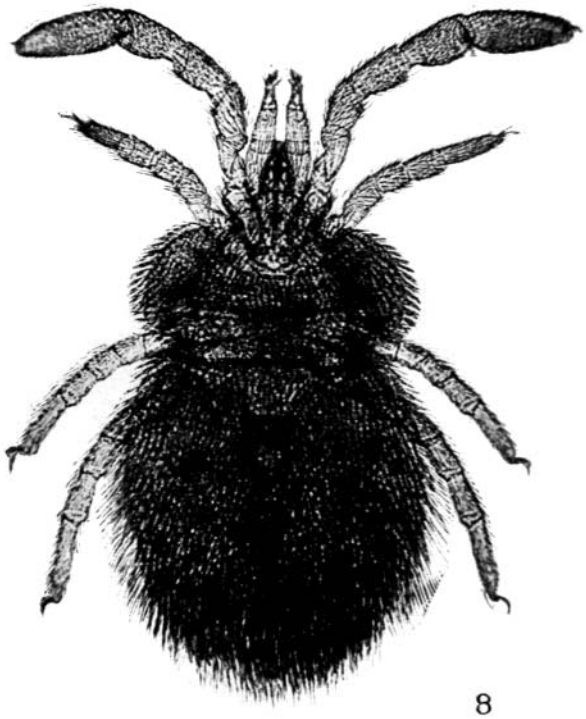
(Nagayo, Miyagawa, Mitamura, and Imamura: The Tsutsugamushi.)



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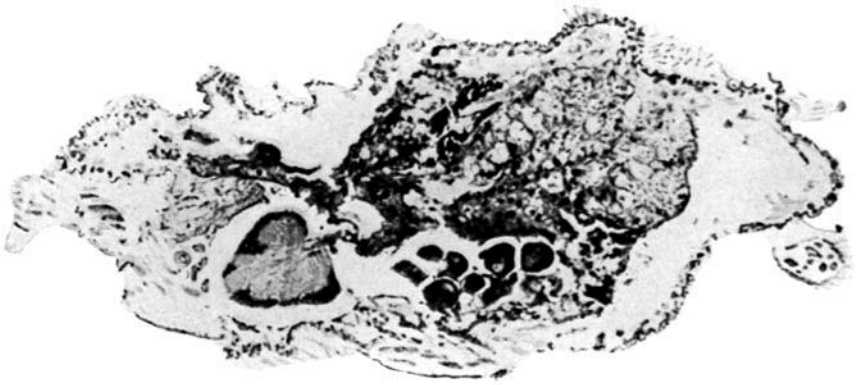
(Nagayo, Miyagawa, Mitamura, and Imamura: The Tsutsugamushi.)



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(Nagayo, Miyagawa, Mitamura, and Imamura: The Tsutsugamushi.)