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Leptotrombidium suzukii (Acari, Trombiculidae): A New Species of Chigger Mite Found on *Apodemus speciosus* (Rodentia, Muridae) on Nakanoshima Island in the Tokara Islands, Kagoshima Prefecture, Japan

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Abstract We describe a new species, *Leptotrombidium suzukii* (Acari, Trombiculidae), of Chigger mites. The type material is laboratory-reared unfed larval trombiculid mites (F6 generation) that developed from the fully engorged larvae collected from *Apodemus speciosus* (Temminck, 1844) from Nakanoshima Island in the Tokara Islands, Kagoshima Prefecture, Japan. The new species is closely related to *Leptotrombidium bunaense* (Womersley, 1952), n. comb., n. stat. [=*Leptotrombidium* (*Leptotrombidium*) *bunaense* Vercammen-Grandjean and Langston, 1976, a new junior synonym and homonym of *Trombicula deliensis* form *bunaensis* Womersley, 1952], but it is distinguished by the arrangement of the dorsal setae and long scutal setae.

Key words: *Leptotrombidium suzukii*, *Leptotrombidium deliense*, *Trombicula* (*Leptotrombidium*) *deliensis* form *bunaensis*, *Leptotrombidium* (*Leptotrombidium*) *bunaense*, Nakanoshima Island.

Introduction

Leptotrombidium deliense (Walch, 1922) is one of the most common vector species of scrub typhus, or tsutsugamushi disease, in Southeast Asia (Traub and Wisseman, 1974). The first case of scrub typhus in Okinawa Prefecture, Japan, was found on Miyakojima Island, Miyako-shi in 2008, and was presumably caused by the vector mite *L. deliense* (Takada *et al.*, 2011; Takada, 2013).

Suzuki (1983) reported the results of intensive surveys of chiggers found on various mammals and reptiles on Takarajima and Nakanoshima Islands, comprising the Tokara Islands, Kagoshima Prefecture, Japan. He collected at least 7000 individuals of one unknown chigger species from *Apodemus speciosus* (Temminck, 1844) (Rodentia, Muridae) on Nakanoshima Island. They were tentatively identified as *L. deliense*. However, slight morphological and ecological differences had been noticed between this species from Nakanoshima and the Southeast Asian *L. deliense* (Suzuki, personal communication).

To elucidate whether the species (Naka-*L. deliense*) collected on Nakanoshima Island is the same species as *L. deliense* (Miyako-*L. deliense*) from Miyakojima Island, Okinawa Prefecture, Japan, we conducted crossbreeding experiments between Naka-*L. deliense* and Miyako-*L. deliense*. In addition, we performed morphological investigations of the scutum in each of the four *L. deliense* populations.

Materials and Methods

Crossbreeding experiments

Chiggers: On July 3, 2012, one of the authors (Noda) collected many engorged Naka-*L.*

deliense larvae from *Apodemus speciosus* on Nakanoshima Island and sent them to the senior author. These larvae were put in a plastic container, 60 mm in diameter and 37 mm in height, containing plaster of paris mixed with charcoal powder at a ratio of 1 : 9 by weight. The chiggers were kept under humid condition at 25°C and fed with fresh eggs from colembolla, *Sinella curviseta* (Brook, 1882) (Collembola, Entomobryidae) (Takahashi *et al.*, 1988). When they developed into adults, their sex was identified with a photomicroscope. Adults of the F5 generation were used for the present experiments.

Leptotrombidium deliense from Miyakojima Island (Miyako-*L. deliense*) were collected from *Rattus rattus* (Linnaeus, 1758) on July 15, 2011, and fully engorged larvae were reared in the laboratory according to the above specifications. Adults of the F4 generation were used for the present experiments.

Crossbreeding: One female Miyako-*L. deliense* was kept with one male Naka-*L. deliense* in a container. Five replicate containers were prepared and kept under the 25°C incubator, and observed for three months to determine if any of the five females laid eggs.

For the reciprocal mating experiment, one male Miyako-*L. deliense* was kept with one female Naka-*L. deliense* in a container. Five replicate containers were maintained and observed under the 25°C incubator conditions, as described above.

For the control experiments, paired male and female Miyako-*L. deliense* were kept together in a container. Five identical containers were used for successive generation. The experiment for Naka-*L. deliense* was also conducted by the same procedure used for Miyako-*L. deliense*.

Morphological investigations

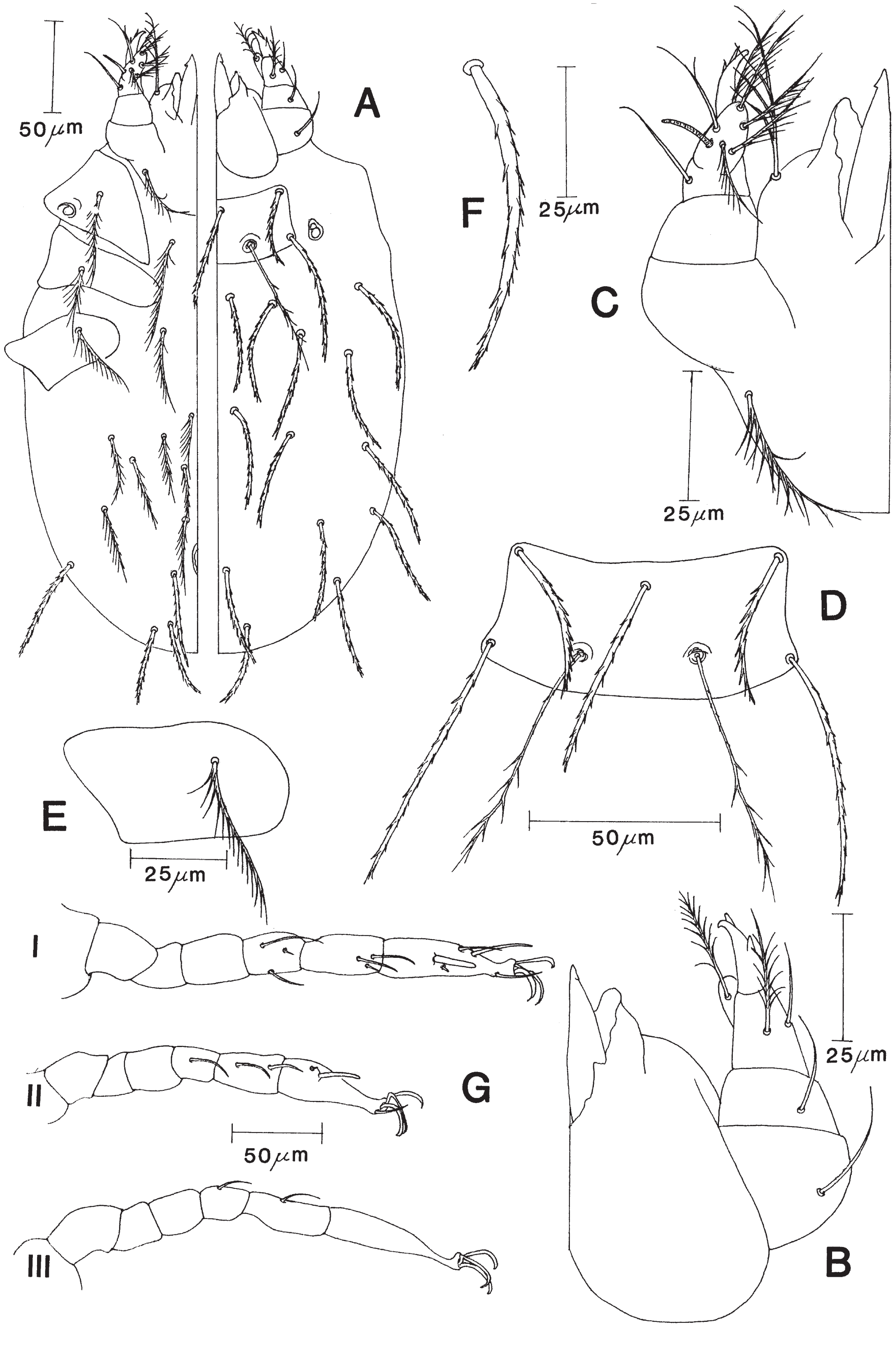
The unfed larval chiggers of Naka-*L. deliense* (F6 generation) and Miyako-*L. deliense* (F5 generation) from the colonies maintained in our laboratory were used. The US Army Medical Research Unit and the Armed Forces Research Institute of Medical Sciences supplied us with the unfed *L. deliense* larvae from Malaysia and Thailand, respectively. Ten unfed larvae from each of the four *L. deliense* populations were mounted in Gum Chloral solution for use in morphological investigations with the photomicroscope.

An independent t-test was used to compare the widths of each anterior scutum (AW) and each posterior scutum (PW) for *L. deliense* on Miyakojima Island, Naka-*L. deliense*, and *L. deliense* from the Malaysian and Thailand populations.

The abbreviations and terminology in this study are consistent with those used by Goff *et al.* (1982), with some modifications: anterolateral seta (AL); anteromedian seta (AM); distance from anterolateral setal base to posterolateral setal base on one side (AP); distance from sensillary bases to extreme anterior margin (ASB); anterior width of scutum (AW); branched seta (B); postanal seta or caudal seta (CS); dorsal idiosomal seta (DS); dorsal setal formula (DSF); coxal setation formula (fCx); palpal setal formula (fPp); scutal formula (fSC); ventral setation formula (fV); humeral seta (H, HS); leg index (IP); nude seta (N); total number of idiosomal setae, excluding coxal setae (NDV); posterolateral seta (PL); distance from sensillary bases to extreme posterior margin (PSB); parasubterminala (pST); posterior width of scutum (PW); length of sensilla (S); distance between sensillary bases (SB); scutal depth (SD): SD=ASB+PSB; synthetic identification formula (SIF); sternal seta (StS); and true ventral seta or preanal seta (VS).

All measurements were obtained, and are listed in this paper, in micrometers (μm), with those measurements for the holotype followed by means and ranges for the type series in parentheses (holotype+9 paratypes). All specimens used for the description in the present study were

Fig. 1. *Leptotrombidium suzukii* sp. nov., insufficiently engorged larva. A, Ventral (left) and dorsal (right) views of larva; B and C, dorsal and ventral views of gnathosoma, respectively; D, scutum; E, coxa III; F, humeral seta; G, legs I, II, and III, showing specialized setae (Suzuki, 1983).


A
B
C
D
E
F
G
I
II
III
50 μm
25 μm
25 μm
25 μm
50 μm
25 μm
25 μm
50 μm

fresh, unfed larvae, maintained in the laboratory.

The specimens used for this study, including the types of the new species, are deposited in the acari collection (Tsukuba) of the National Museum of Nature and Science, Tokyo, Japan (NSMT-Ac).

Results

In the control experiment, all females of both species, Miyako-*L. deliense* and Naka-*L. deliense*, successfully laid eggs. However, none of the five Miyako-*L. deliense* (♀) females mated with Naka-*L. deliense* (♂) males laid eggs. In addition, none of the five females mated reciprocally laid eggs. Based on these results, Naka-*L. deliense* is considered to be reproductively isolated from Miyako-*L. deliense*. The results of the independent t-tests for the measurements of each AW and PW are shown in Table 1. There was a significant difference between AW and PW of Naka-*L. deliense* and those of the three other populations of *L. deliense*. In addition, the posterolateral setae of Naka-*L. deliense* were much longer than the setae of each of three other populations (t-test, $p<0.05$) (Fig. 2). Therefore, "Naka-*L. deliense*" is considered to be a different species from the *L. deliense* from Malaysia, Thailand, and Miyakojima Island of Japan.

We propose that Naka-*L. deliense* is a new species of the genus *Leptotrombidium* according to (1) the results mentioned above, (2) the morphological differences based on the standard data of the known *L. deliense* species including *Trombicula (Leptotrombidium) deliensis* form *bunaensis* Womersley, 1952 collected from various localities of Southeast Asia (Womersley, 1952; Vercammen-Grandjean and Langston. 1976), and (3) the chromosomal number of Naka-*L. deliense* (2n=14), which is different from the chromosomal number (2n=16) of the Miyako- *L. deliense* population (Tsurusaki and Takahashi, 2014).

Family Trombiculidae

Genus ***Leptotrombidium*** Nagayo, Miyagawa, Mitamura and Imamura, 1916

Leptotrombidium suzukii sp. nov.

[New Japanese name: Suzuki-tsutsugamushi]

(Figs. 1, 2D)

Leptotrombidium deliense (not of Walch, 1922): Suzuki, 1983: 9, Fig. 2.

Diagnosis of larvae. SIF=7B-B-3-2111.0000; fPp=NN/BNN/7B; fCx=1.1.1; IP=825 (824, 804–844); pST=N; fSC=PL≫AM>AL; 2 humeral setae; DSF=2H, 8, 6, 2, 6, 4, 2=30 (holotype); 2H, 8, 6, 6, 4, 2=28; 2H, 8, 6, 4, 4, 2=26 (Fig. 1); DS=26–30 (30 in holotype); fV=2, 2, 8, 6, 2 U 4,

Table 1. The results of independent t-test of the length of anterior width of scutum (AW) and posterior width of scutum (PW) of each *L. deliense* collected at four localities such as Malaysia, Thailand, Miyakojima Island of Japan and Nakanoshima Island of Japan.

AW

Localities	Malaysia	Thailand	Miyakojima	Nakanoshima
Malaysia		0.9	0.27	0.0001*
Thailand			0.33	<0.0001*
Miyakojima				<0.0001*
Nakanoshima				

PW

Localities	Malaysia	Thailand	Miyakojima	Nakanoshima
Malaysia		0.14	0.02*	0.001*
Thailand			0.16	<0.0001*
Miyakojima				<0.0001*
Nakanoshima				

*: Significant differences ($P<0.05$) were observed.

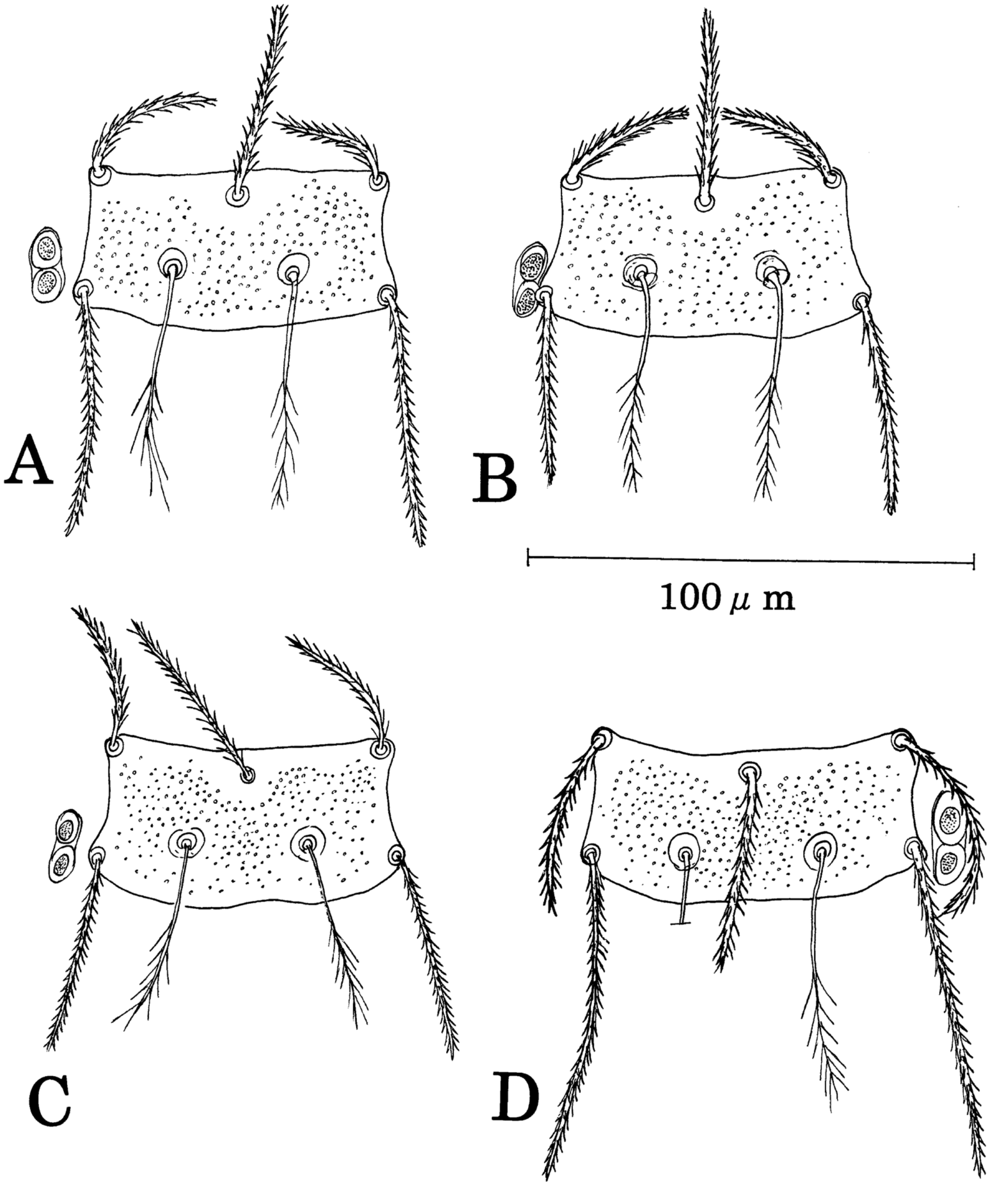


Fig. 2. Scutum of *Leptotrombidium* spp. A–C, *L. deliense* (A, Thailand; B, Malaysia; C, Miyakojima Island in Japan); D, *L. suzukii* sp. nov. (Nakanoshima Island in Japan).

4, 2=30 (holotype); 2, 2, 6, 4, 4 U 4, 4=26 (Fig. 1); NDV=52–61 (56 in holotype).

Type specimens. Holotype (NSMT-Ac 14149) is an unfed larva from the F6 generation, reared in the laboratory, originating from the fully engorged larvae collected by Shinichi Noda from *Apodemus speciosus* on Nakanoshima Island (29°51′38″N, 129°52′48″E), Toshima-mura, comprising the Tokara Islands, Kagoshima Prefecture.

Paratypes (NSMT-Ac 14150–14158) consist of nine unfed larvae from the same generation, host, location, and collection date as the holotype collected by Shinichi Noda.

Other collection records. Nakanoshima Island, Toshima-mura, Kagoshima-gun, Kagoshima Prefecture, Japan are as follows: (1) One of the authors (Shinichi Noda) collected 300 engorged larvae from 10 *A. speciosus*, and 16 engorged larvae from 2 *Rattus rattus* 6-VIII-2005, 132 engorged larvae from 3 *A. speciosus* 17-VI-2009, and 162 engorged larvae from 4 *A. speciosus* 1-VII- 2012.

(2) Dr. Masaharu Motokawa captured many *A. speciosus* and kept them in the 70% ethanol in the Kyoto University Museum. One of the authors (Takahashi) collected *L. suzukii* sp. nov. from them: 9 engorged larvae from *A. speciosus* (KUZ-M-2030), 3-VII-1998; 56 engorged larvae from KUZ-M-2033, 3-VII-1998; 38 engorged larvae from KUZ-M-2032, 3-VII-1998; 1 engorged larva from KUZ-M-2031, 3-VII-1998; 1 engorged larva from KUZ-M-2039, 3-VII-1998; 8 engorged larvae from KUZ-M-2038, 4-VII-1998; 17 engorged larvae from KUZ-M-2036, 4-VII-1998; 23 engorged larvae from KUZ-M-2626, 26-XI-1999; 5 engorged larvae from KUZ-M-2624, 26-XI-1999; 4 engorged larvae from KUZ-M-2622, 26-XI-1999; 15 engorged larvae from KUZ-M-2617, 26-XI-1999; 5 engorged larvae from KUZ-M-2621, 26-XI-1999; 33 engorged larvae from KUZ-M-2618, 26-XI-1999; 42 engorged larvae from KUZ-M-2625, 26-XI-1999; 12 engorged larvae from KUZ-M-2620, 26-XI-1999; 21 engorged larvae from KUZ-M-2616, 26-XI-1999; 12 engorged larvae from KUZ-M-2619, 26-XI-1999; 16 engorged larvae from KUZ-M-2615, 26-XI-1999; 7 engorged larvae from KUZ-M-2623, 26-XI-1999; 29 engorged larvae from KUZ-M-2630, 27-XI-1999; 5 engorged larvae from KUZ-M-2628, 27-XI-1999; 25 engorged larvae from KUZ-M-2632, 27-XI-1999; 18 engorged larvae from KUZ-M-2627, 27-XI-1999; 42 engorged larvae from KUZ-M-2631, 27-XI-1999; 26 engorged larvae from KUZ-M-2629, 27-XI-1999.

Description of larvae.

Color: The live, unfed larvae were pale red in color; the fully engorged larvae were pale yellow.

Idiosoma: Body longer than wide, measuring 226 (225, 207–242) long and 187 (192, 187–198) wide. Two pairs of eyes on the ocular plates, located by scutum at the level of a slightly upper part of PL bases; diameter of anterior and posterior eyes 10 (9, 8–10) and 9 (8, 8–9), respectively.

Gnathosoma: Gnathosomal base moderately punctated, 70 (72, 70–74) wide at the level of the bases of a pair of branched setae; cheliceral bases triangular and posterior margin slightly rounded, 46 (45, 44–46) long by 41 (39, 34–42) wide; cheliceral blade 34 (37, 34–40) long by 8 (9, 8–10) wide, with tricuspid cap; Galeala branched. fPp=NN/BNN/7B; palpal claw stout 17 (17, 16–19) long by 4 (4, 3–4) wide, three pronged, with axial prong 12 (12, 12–13), two accessory prongs of almost equal length.

Scutum: Shape rectangular, wider than long, PW/SD ratio 2.0 (2.0, 1.9–2.0) with anterior margin slightly concave; lateral margins slanting outwards with slight concavity, posterior margin slightly rounded posterior to level of PL setae; posterior corners somewhat extended; width of scutum greatest at PL corners; AL setae situated on the anterior corners; PL setae situated on the posterior corners; AM seta base slightly below AL seta bases; relative length of the scutal setae, PL≫AM>AL; AL and AM setae with a moderate number of slightly fine setules for almost their entire length; PL setae barbed with a moderate number of stout and short setules for almost their entire length, resembling humeral setae and dorsal idiosomal setae in appearance; each sensillary base round with several small ridges; sensillary bases in line with or slightly anterior to level of PL bases; sensillae flagelliform nude basally with setules on distal three-quarters; small granular punctations distinctly distributed on scutum, except around AM base and posterior portions of sensillary bases. Scutal measurements: AW, 67 (69, 68–72); PW, 75 (76, 75–78); SB, 31 (32, 31–34); ASB, 26 (27, 25–29); PSB, 12 (12, 11–14); AP, 26 (26, 16–28); AM, 58 (58, 58–59); AL, 46 (47, 47–49); PL, 75 (73, 71–75); S, 68 (68, 66–69).

Idiosomal setae: One pair HS, measuring 55 (56, 55–57); 26–30 arranged in irregular rows;

2H, 8, 6, 2, 6, 4, 2=30 (holotype); 2H, 8, 6, 6, 4, 2=28; 2H, 8, 6, 4, 4, 2=26 (Fig. 1). Humeral and dorsal idiosomal setae are covered with a moderate number of thick and short setules for almost their entire length, similar to PL setae, but not similar to AM and AL setae on the scutum. Length of dorsal setae as follows: medial seta of first post-humeral row 50 (49, 48–50); dorsal medial seta in central position 48 (49, 48–49); posterodorsal medial seta 46 (49, 46–53); dorsal terminal seta 47 (48, 46–51); StS 2-2 [anterior 54 (52, 48–54), posterior 40 (41, 40–42)], covered with a moderate number of fine setules on their entire length, more pliant than preanal setae; 18–20 preanal setae and 8–10 postanal setae (20 and 10 setae in holotypes, respectively) similar in nature to StS but shorter; length of medial seta in first preanal setal row 32 (33, 32–35); medial seta in first postanal setal row 50 (52, 50–55), different in nature from dorsal idiosomal setae, but shorter; total number of idiosomal setae, excluding coxal setae, 52–60 (60 in holotype, 52 in Fig. 1).

Leg: IP=825 (824, 804–844). All 7-segmented, terminating in a pair of claws and a slender claw-like empodium. Onychotriches lacking. Conspicuous small punctations on coxae and free leg segments. No modified leg segments.

Leg I: 282 (274, 256–285) long; tarsus+ pretarsus 65 (66, 65–68) long by 24 (26, 24–27) wide; coxa with 1B; trochanter 1B; basifemur 1B; telofemur 5B; genu 4B, 2 genualae [dorsal genuala 24 (26, 24–28), distal genuala 25(26, 25–26)], microgenuala 5 (4, 4–5); tibia 8B, 2 tibialae [proximal tibiala 21 (21, 20–21), distal tibiala 22 (20, 19–22)], microtibiala 4 (4, 4–5); tarsus 21B, tarsala 19 (19, 18–19) located on 1/2 of segment, microtarsala 4 (4, 4–5), a nude subterminala 28 (28, 27–28), a short parasubterminala 8 (9, 8–10), pretarsala 16 (17, 16–17).

Leg II: 252 (256, 252–260) long; tarsus+ pretarsus 55 (56, 55–57) long by 22 (22, 22–23) wide; coxa 1B; trochanter 1B; basifemur 2B; telofemur 4B; genu 3B, one genuala 19 (21, 19–23); tibia 8B, 2 tibialae [proximal tibiala 18 (17, 17–18), distal tibiala 20 (18, 16–20)]; tarsus 16B, tarsala 18 (17, 16–18), microtarsala 4 (4, 3–4), pretarsala 17 (16, 15–17).

Leg III: 291 (294, 291–299) long; tarsus+ pretarsus 74 (76, 74–78) long by 18 (18, 17–19) wide; coxa 1B; trochanter 1B; basifemur 2B; telofemur 3B; genu 3B, one genuala 20 (21, 19–25); tibia 6B, tibiala 24 (24, 23–26); tarsus 15B.

Distribution. Nakanoshima Island, Tokara Islands, Kagoshima Prefecture, Japan.

Etymology. This species is dedicated to Dr. Hiroshi Suzuki, formerly of the Institute of Tropical Medicine, Nagasaki University, in recognition of his initial discovery of this new species and also in recognition of his substantial contribution to our knowledge of the chigger fauna on the Nansei Archipelago of Japan.

Remarks. Chiggers of the genus *Leptotrombidium* Nagayo, Miyagawa, Mitamura and Imamura, 1916 have a worldwide distribution, and large numbers (>350) of species have been recorded from various areas around the world (Womersley, 1952; Sasa, 1956; Vercammen-Grandjean and Langston, 1976; Domrow and Lester, 1985; Li, 1997; Kudryashova, 1998; Fernandes and Kulkarni, 2003; Stekolnikov, 2013). Because they closely resemble each other, identification of a species belonging to this genus is sometimes very difficult because of their considerable morphological variations. *Leptotrombidium deliense*, which is the main vector species of scrub typhus in Southeast Asia, is especially hard to identify. This species is common and widely distributed, ranging from Miyakojima Island, southern Japan in the north, to northern Australia in the south, and to Pakistan in the west (Womersley, 1952; Traub and Wisseman, 1967; Vercammen-Grandjean and Langston, 1976; Takada *et al.*, 2011; Takada. 2013).

According to Womersley's (1952) standard data for 24 populations of *L. deliense*, the dorsal scutum tends to increase in size along a west to east gradient, with the populations from New Guinea and Northern Queensland having somewhat larger scutum width than populations farther east. The population from Buna, New

Guinea, is significantly different from all the others in the length of the scutal setae, especially PL setae, which had a mean length of 85 µm. Therefore, Womersley (1952) proposed that this population was a distinct, possibly local, form with the name *Trombicula* (*Leptotrombidium*) *deliensis* form *bunaensis*. His paper provided standard morphological data of the scutum and figures of the above-mentioned new form. This scientific name for a form is originally valid and is regarded as a subspecies of *T.* (*L.*) *deliensis* in accordance with Article 45.6.4 of the International Code of Zoological Nomenclature (International Commision on Zoological Nomenclature, 1999). Thereafter, Vercammen-Grandjean and Langston (1976) described a new species "*Leptotrombidium* (*Leptotrombidium*) *bunaense* n. sp." on the basis of the same specimen as holotype which Womersley used for the description of the new form, probably because they misunderstood that the Womersley's form name should be invalid. After careful consideration of the morphological characteristics, we regard *T.* (*L.*) *deliensis* form *bunaensis* Wormersley, 1952 as an independent species of the genus *Leptotrombidium*, and state herein as *L. bunaense* (Wormersley, 1952), n. comb., n. stat. Although *L.* (*L.*) *bunaense* Vercammen-Grandjean and Langston, 1976 is also a valid name, it is regarded as a new junior synonym and homonym of the Wormersley's species.

Although *L. suzukii* sp. nov. resembles each population of *L. deliense* from Thailand, Malaysia, and Miyakojima Island of Japan, *L. suzukii* is easily distinguished by the size of the scutum, situation of the sensillary bases, and the long posterolateral setae of the scutum (Fig. 2).

Leptotrombidium suzukii is also closely related to *L. bunaense* in the combination of characters mentioned in the analysis. However, *L. suzukii* can be distinguished from *L. bunaense* by the following characteristics: In *L. suzukii*, DS are 2–8–6–(2)–6 (4)–4–2=26–30 (DS of *L. bunaense* 2–8–6–6–8–6–4–2=42); Length of PL is 73 (85); Length of humeral setae 56 (78).

Biogeographic distribution. Within the Tokara Islands, Watase's line, which is between Akusekijima Island and Kodakarajima Island, marks a major bio-geographic boundary. The Palaearctic subregion is north of this line, while south of the line is the northern limit of the Oriental subregion. Miyakojima Island, where *Leptotrombidium deliense* is distributed, is the northern limit of the Oriental subregion, whereas Nakanoshima Island, where *L. suzukii* sp. nov. is distributed, is situated within the Palaearctic subregion. This island is also the southern limit of *Apodemus speciosus* (Kaneko, 2005). It is possible that *L. suzukii* is distributed on a few islands within the Palaearctic subregion.

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