



# Identification of Trombiculid Chigger Mites Collected on Rodents from Southern Vietnam and Molecular Detection of Rickettsiaceae Pathogen

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**Abstract:** Trombiculid “chigger” mites (*Acar*) are ectoparasites that feed blood on rodents and another animals. A cross-sectional survey was conducted in 7 ecosystems of southern Vietnam from 2015 to 2016. Chigger mites were identified with morphological characteristics and assayed by polymerase chain reaction for detection of rickettsiaceae. Overall chigger infestation among rodents was 23.38%. The chigger index among infested rodents was 19.37 and a mean abundance of 4.61. A total of 2,770 chigger mites were identified belonging to 6 species, 3 genera, and 1 family, and pooled into 141 pools (10-20 chiggers per pool). Two pools (1.4%) of the chiggers were positive for *Orientia tsutsugamushi*. *Rickettsia* spp. was not detected in any pools of chiggers. Further studies are needed including a larger number and diverse hosts, and environmental factors to assess scrub typhus.

**Key words:** *Orientia tsutsugamushi*, *Rickettsia* sp., chigger mite, ectoparasite

## INTRODUCTION

Trombiculid mites (*Acar*: *Trombiculidae*) are ectoparasites that are found in grasses and herbaceous vegetation. The larval mites (chiggers) feed blood on rodents and another animals and birds. More than 3,000 species of chiggers have been identified, but only about 20 species frequently attack humans, and the most relevant species e.g., *Eutrombicula alfreddugesi* in North and South America, *Neotrombicula autumnalis* in Europe, and *Leptotrombidium* spp. in Asia, that are considered to play an important role in transmission of *Orientia tsutsugamushi*.

The life cycle of Trombiculid mites include 7 stages of active and inactive stages. The larval stage chigger is the only stage of development that parasitizes a wide range of animals, including rats, squirrels, shrews, and birds. Humans are accidentally infected with *O. tsutsugamushi* through bites of chigger mites in grassy fields, in open and forested areas. The bite sites are on the skin, especially thin and clothing constricts.

*Orientia tsutsugamushi* is a gram-negative bacteria and causative agent of scrub typhus, is a vector-borne zoonotic disease with the potential of causing life-threatening febrile infection in humans. *Orientia tsutsugamushi*, is maintained in the Trombiculid mites through transovarial transmission. Scrub typhus is endemic in South and East Asia regions with more than one billion people at risk with approximately one million new cases reported annually.

There was limited surveillance. Surveys on the species composition of chigger mites were conducted at disperse sites in southern as well as throughout Vietnam. Study on pathogens in chigger mites have not been performed in southern Vietnam.

We performed this cross-sectional study to identify a species composition of chigger mites and presence of rickettsiaceae bacteria, especially *Orientia tsutsugamushi* in the mites parasitized in rodents collected in southern Vietnam.

## MATERIALS AND METHODS

### Site description

Seven ecosystems in southern Vietnam including Bu Gia Map National park (Binh Phuoc province), Can Gio Man-

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grove Forest (Ho Chi Minh City), Lang Sen Wetland Reserve (Long An province), Lung Ngoc Hoang Nature Reserve (Hau Giang province), Cat Tien National Park (Dong Nai province), U Minh Ha National Park (Ca Mau province), U Minh Thung National Park (Kien Giang province).

#### Rodent collection

Rodent were collected from July to October 2015 (rainy season) and March to April 2016 (dry season). A total of 70 Sherman collapsible live capture traps (H. B. Sherman Traps, Inc., Tallahassee, Florida, USA) at each of the sites were set each night (from 16:00 to 05:00 the following day). Traps were baited with fried fish, potatoes or other local food. Rodents were removed from the traps and placed individually in a white cloth bag labeled with the time of collection, date and an unique collection that identified the survey site and other pertinent information, and then transported to laboratory of Ectoparasitology, Department of Parasitology and Entomology, Ho Chi Minh Institute of Malariology.

Rodents were euthanized at the laboratory of Ectoparasitology Department according to the protocol of Ho Chi Minh Institute of Malariology, Parasitology and Entomology with ketamine (100 mg/kg) and xylazine (10 mg/kg) was intraperitoneally injected at dosage of 0.2 ml/100 gm body weight. Rodents were identified to species using morphology and taxonomic keys.

All animals received a good care by well-trained staffs. The study protocols were performed according to the ethical guidelines for care and use of experimental animals approved by the Ethical committee board of Ho Chi Minh Institute of Malariology, Parasitology and Entomology (Permit number 034/2015/HCMIMPE-IRB).

All personnel were trained on safety measures when conducting investigations, collection, treatment and identification. If participants developed infectious diseases (occupational diseases), they were entitled to benefits and regimes according to current regulations.

#### Chigger mites collection

Rodents were carefully examined e.g., the groin, leg, anal area, ear, nose, eyelid, and crest by brushing these areas with fine combs. Then chigger mites were collected into plastic vials containing 70% ethanol and labeled individually with an unique number assigned to each of survey sites.

#### Chigger identification

The chigger mites collected from rodents were identified based on morphologic criteria following taxonomic keys. The chiggers were mounted on glass slides with Hoyer's medium and observed under a binocular microscope at x400 magnification. The key for identification of chigger mite species was based on documents of Van Chau Nguyen. Mites were put on a pool of 10-20 individuals according to their genera and host species. Stored at 4°C for pathogen identification.

#### Rickettsiaceae detection

The genomic DNA was extracted from the chigger mite pools using the ISOLATE II Genomic DNA Kit (Cat. BIO-52066, Bionline, UK) according to the manufacturer's instructions. A final elution volume of 50 µl DNA samples were aliquoted into 2 eppendorf tubes and store at -80°C until used.

A multiplex PCR method was performed for the detection and differential diagnosis of *Oriental tsutsugamushi* and *Rickettsia* spp. based on 2 primers sets using groEL gene sequences.

In brief, a duplex PCR was run with total reaction volume of 50 µl, containing 25 µl 2x MyTaq HS Mix 2x (Bionline, UK); 2.0 µl Multiplex Primer 25 nmol/l; 20 µl distilled water and 3 µl of DNA template. PCR cycle: 94°C for 5 minutes, followed by 45 cycles: 94°C for 30 sec, 56°C for 30 seconds, and 72°C for 45 seconds and ended at 72°C for 7 minutes (Applied Biosystem 2720 Thermocycle; Applied Biosystem, Foster City, California, USA).

All PCR amplicons were electrophorized in 2% agarose containing ethidium bromide. Bacteria species were identified based on amplicon size.

#### Data processing and analysis

The epidemiological parameters such as mean abundance of chiggers (total number of chiggers collected divided by total number of rodents), chigger infestation rate (percentage of rodents infested with chiggers), and chigger index (total number of chiggers divided by total number of infested rodents) were calculated using SPSS software, version 16.0 (SPSS, Chicago, Illinois, USA). Map was drawn using ArcView GIS software, version 3.1 (ESRI).

## RESULTS

A total of 600 rodents belonging to 8 species were captured at 7 sites during 2015 to 2016 (Table 1). Overall, *Rattus norvegicus*

**Table 1.** Number of rodents infected with chigger mites at study sites in southern Vietnam, 2015-2016

Rodent	No. rodents infested with chiggers/total trapped rodents							Subtotal (%)
	CG	LS	LNH	BGM	CT	UMT	UMH	
<i>Suncus murinus</i>	0/10	0/2	0/13	0/5	0/10	0/3	0/10	0/53 (0)
<i>Ratus norvegicus</i>	4/17	11/14	6/26	4/29	6/18	9/26	7/13	47/143 (32.87)
<i>R. argentiventer</i>	0/0	9/25	7/24	0/0	0/1	4/15	4/25	24/90 (26.67)
<i>R. fulvescens</i>	3/14	7/12	2/14	2/9	0/17	12/34	7/13	33/113 (29.20)
<i>R. edwardsi</i>	1/10	2/2	1/12	2/18	5/14	12/28	4/14	27/98 (27.55)
<i>R. molliculus</i>	1/9	2/22	2/9	2/8	0/11	0/11	1/8	8/78 (10.26)
<i>Mus musculus</i>	0/2	0/2	0/5	0/2	0/0	0/1	0/2	0/14 (0)
<i>R. rattus</i>	0/0	0/0	0/0	2/5	1/5	0/0	1/1	4/11 (36.36)
Total	9/62	31/79	18/103	12/76	12/76	37/118	24/86	143/600 (23.38)

CG, Can Gio Mangrove Forest; LS, Lang Sen Wetland Reserve; LNH, Lung Ngoc Hoang Nature Reserve; CT, Cat Tien National Park; UMH, U Minh Ha National Park; UMT, U Minh Thuong National Park.

**Table 2.** Species composition and distribution of chigger mites infested on rodents in southern Vietnam, 2015-2016

Chigger mite	No. of chiggers	Rodent species (host)
<i>Ascoschoengastia (Laurentella) indica</i>	448	<i>Rattus fulvescens</i> , <i>R. edwardsi</i> , <i>R. argentiventer</i> , <i>R. norvegicus</i> , <i>R. rattus</i>
<i>Gahrliepia (Walchia) chinensis</i>	678	<i>R. edwardsi</i> , <i>R. fulvescens</i> , <i>R. molliculus</i>
<i>Gahrliepia (Walchia) lupella</i>	299	<i>R. argentiventer</i> , <i>R. norvegicus</i>
<i>Gahrliepia (Walchia) parapacifica</i>	111	<i>R. molliculus</i>
<i>Leptotrombidium (Leptotrombidium) deliense</i>	1,148	<i>R. edwardsi</i> , <i>R. argentiventer</i> , <i>R. norvegicus</i> , <i>R. molliculus</i> , <i>R. fulvescens</i> , <i>R. rattus</i>
<i>Leptotrombidium (Leptotrombidium) striatum</i>	86	<i>R. fulvescens</i> , <i>R. edwardsi</i> , <i>R. rattus</i>
Total	2,770	

*cus* was the most commonly collected rodent (43), followed by *R. fulvescens* (113), *R. edwardsi* (98), *R. argentiventer* (90), *R. molliculus* (78), *Suncus murinus* (53), *Mus musculus* (14), *R. rattus* (11).

A total of 143 rodents belonging to six species infested with chiggers (infestation rate = 23.38%). *Suncus murinus* and *Mus musculus* were not infested with chigger mites. *Rattus rattus* had the highest infection rate 36.36%, followed by *R. norvegicus* 32.87%, *R. fulvescens* 29.20%, *R. edwardsi* 27.55%, *R. argentiventer* 26.67%, and *R. molliculus* 10.26% (Table 1).

#### Composition and distribution of Chigger mites

A total of 2,770 chigger mites were collected from 143 rodents with an overall chigger index of 19.37 and a mean abundance of chiggers 4.61.

Chigger mites belonged to 6 species *Ascoschoengastia (Laurentella) indica*; *Leptotrombidium (Leptotrombidium) deliense*, *Leptotrombidium (Leptotrombidium) striatum*; *Gahrliepia (Walchia) chinensis*, *Gahrliepia (Walchia) lupella*, *Gahrliepia (Walchia) parapacifica*, belonging to 3 genera, and 1 family. *L. (L.) deliense*, *G. (W.) chinensis* and *As. (L.) indica* were the most commonly collected species (Table 2).

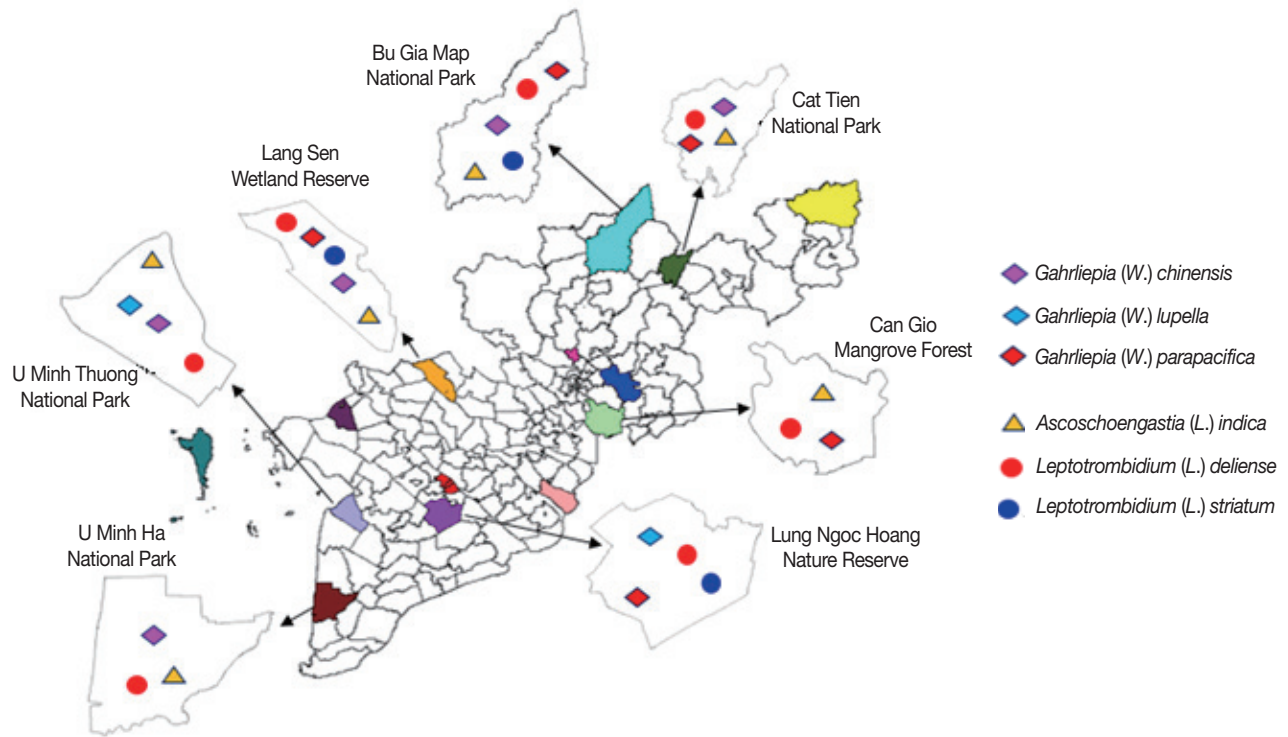
The results show that *R. norvegicus*, *R. fulvescens*, *R. edwardsi* and *R. rattus* were the main hosts of 6 chigger mite species, especially *Leptotrombidium* spp. The remaining rodents were the hosts of some other species. The distribution of chigger mites parasitizing on rodents according to the survey sites were shown in Fig. 1.

The geographical distribution showed that *L. (L.) deliense* was collected from rodents at all surveyed sites, while *As. Indica* was collected at 6 sites, *G. (W.) chinensis* and *G. (W.) parapacifica* at 5 sites. The remaining chigger mite species showed narrow distribution, e.g., *G. (W.) lupella* was found at 2 survey sites and *L. (L.) striatum* at 3 sites.

#### Pathogens in chigger mites

The results showed that 1.4% pools of chigger mites were positive with *Orientia tsutsugamushi* (Fig. 2). One sample of *Ascoschoengastia (L.) indica* was infected *O. tsutsugamushi* (4.35%). One pool of *L. (L.) deliense* was infected *O. tsutsugamushi* (1.72%). There were not a positive pool for *Rickettsia* spp. (Table 3).

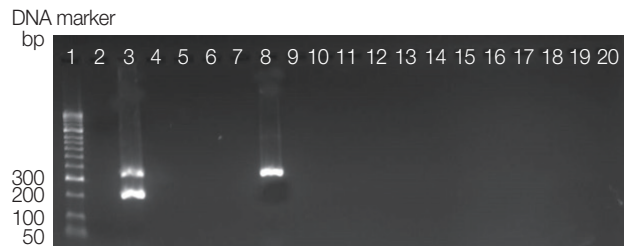
At 7 survey sites, an *O. tsutsugamushi* -positive mite pool was found in Bu Gia Map National Park, Binh Phuoc province belonged to *As. (L.) indica* species (parasitized on *R. argentiventer*).



**Fig. 1.** Geographical distribution of chigger mites at 7 survey sites in southern Vietnam, 2015–2016.

**Table 3.** Detection rate of rickettsiae in chigger mites by PCR

Chigger mite	No. of pools (individuals)	No. of pool (%) positive with <i>Rickettsia</i> spp.	No. of pool (%) positive with <i>O. tsutsugamushi</i>
<i>As. (L.) indica</i>	23 (448)	0	1 (4.3)
<i>G. (W.) chinensis</i>	34 (678)	0	0
<i>G. (W.) lupella</i>	15 (299)	0	0
<i>G. (W.) parapacifica</i>	6 (111)	0	0
<i>L. (L.) deliense</i>	58 (1,148)	0	1 (1.7)
<i>L. (L.) striatum</i>	5 (86)	0	0
Total	141 (2,770)	0	2 (1.4)



**Fig 2.** Amplicons of duplex PCR detecting DNA of *O. tsutsugamushi* and *Rickettsia* spp. Lane 1, DNA size marker; 2, negative; 3, positive of *Rickettsia* spp. (299 bp) and *O. tsutsugamushi* (366 bp); Lanes 4-20, chigger mite sample pools.

The other one was *L. (L.) deliense* parasitized on *R. norvegicus* host collected in U Minh Ha National Park, Ca Mau province (Fig. 3).

### DISCUSSION

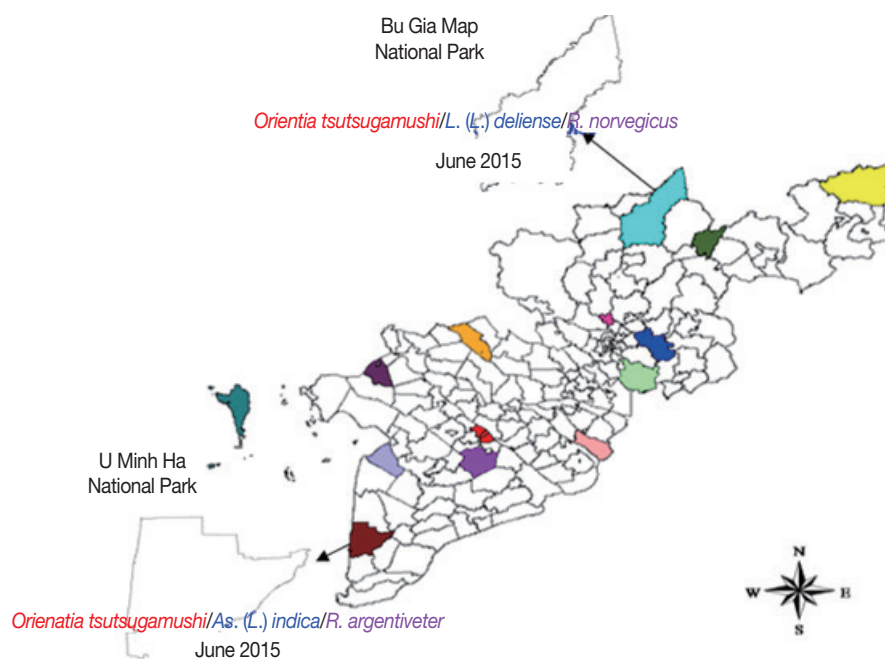
Trombiculid mites’ habitat is prevalent in an area of overgrown fields, wooded, grassy and herbaceous vegetation. More than 3,000 species of chiggers are known in the world, about 350 species have been described in Southeast Asia .

Chiggers mites (family *Trombiculidae*) are considered to be

common ectoparasites of rodents collected from the Mekong Delta, in contrast to gamasid mites, fleas, ticks, and lice are infrequently collected. In our study, 5 per 7 survey sites are in Mekong Delta area, and were forested reserves and parks with abundance of trees, high humidity, lower temperature, light intensity, moist soil and covered with vegetation. It is the ideal environment for the living of trombiculid mites. In the other manner, due to the present human activities, the predator mammals as well as raptorial birds are reduced and the numbers of general species such as squirrels and wild birds increased.

The Central South–Southern Region was the region with the





**Fig 3.** Geographical distribution of rickettsiae in the chigger mites parasitizing on rodents at 7 survey sites in southern Vietnam, 2015-2016.

highest number of chigger mite genus and species with 62 species (58.5% of total chigger mite species in Vietnam), 17 genera, and 2 sub-families. Of them, *Leptotrombidium* and *Gahrliepia* genera were dominant.

Our result had a lower species composition than other studies that were carried out in different regions of Vietnam such as provinces of Quang Binh and Ha Tinh, 16 species; Con Dao Island, 7 species; Quang Nam and Kon Tum, 6 species. In our study, the chigger mites were only collected from rats, while other studies were retrieved them from rodent host groups (wild birds, rabbits, squirrels, raising birds, garbage and animal nests). Of which, 6 species were collected in the rainy season and 5 species were in the dry season. *Leptotrombidium* (*L.*) *striatum* was only collected in the rainy season (July to October) that corresponded to the increasing number of hospitalized scrub typhus patients begun in late August, peaked in October, and then decreased in December.

This hypothesis was further supported by the molecular DNA detection of *O. tsutsugamushi* positive in 1.4% of the mite pools that collected from rodents in the southern region. Two chigger mite species infected with *O. tsutsugamushi* were *L. (L.) deliense* and *As. (L.) indica*. The chiggers of *Leptotrombidium* spp. are vectors of *Orientia tsutsugamushi*, the causative agent of scrub typhus.

In Vietnam, among the chigger mites species discovered,

there were 5 species, acting as vector of scrub typhus, e.g. *L. (L.) deliense*, *L. (L.) akamushi*, *L. (L.) scutellare*, *As (L.) audyi*, and *As. (L.) indica*. In addition, *G. (G.) pintanensis* and *G. (G.) parapacifica* have isolated *O. tsutsugamushi*.

The chigger mite infestation rate of *O. tsutsugamushi* in this study was 1.4% at 7 survey sites and that was lower than 2.7% at 9 survey sites in Korea, and 17.7% in Shandong province, China.

This study provided specific data for the species composition, and distribution of chigger mites infested rodents in southern Vietnam and determined the presence of *O. tsutsugamushi* infection. It is very helpful in development of monitoring strategy, prevention and control of scrub typhus transmitted through chigger mites infested in the rodents.

Further studies are needed with a larger number of host sample size, diversity of environmental condition to give the overall geographic distribution and assess the full risk of human exposure to *Trombiculid* "chigger" mites.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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