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Abundance and taxonomic characterization of chigger mites (Acari: Trombiculidae and Walchiidae) associated with rodents in selected scrub typhus-prone areas in Southern and Western provinces of Sri Lanka

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Abstract

Larval trombiculid mites (chiggers) are the vectors and reservoirs of the potentially lethal infectious disease, scrub typhus (ST) caused by *Orientia tsutsugamushi*. Small rodents are natural hosts of parasitic larval stage of the chigger mites. This study focused on determining the abundance of chigger mites associated with rodents in Sri Lanka and the taxonomic characterization of field-caught chiggers. Field sampling was conducted in the districts of Galle, and Hambantota of the Southern Province, and Gampaha of the Western Province, in 2019 and 2020. Sampling sites were selected according to the patient distribution. A total of 58 small mammals (rodent species: *Rattus rattus* (Black rat), *Rattus norvegicus* (Brown rat), *Tatera indica* (Indian gerbil), *Gollunda ellioti* (Indian bush rat) and *Suncus murinus* (Asian house shrew)) were captured using baited traps. Chiggers from the captured rodents were speciated morphologically by visual inspection and morphometry using a camera-mounted light microscope (x100). A total of 394 chigger mites were collected. Three different genera were identified, including *Leptotrombidium*, *Schoengastiella*, and *Microtrombicula*. *Leptotrombidium imphalum* (72.59%; n=286) was the predominant species, followed by *Schoengastiella punctata* (8.12%; n=32). Some specimens were identifiable only up to genus level, *Leptotrombidium* sp. (3.55%; n=14) and *Microtrombicula* sp. (4.82%; n= 19). Some (7.11%; n=28) were not trombiculid mites, while 3.81%; n=15 was damaged beyond identification. *Leptotrombidium imphalum* was detected for the first time parasitizing the murids - *Rattus novergicus* and *Tatera indica* in the district of Galle, a new locality. In addition, *S. punctata* was recorded in a new locality in the Gampaha district, Western province with a new host association, *Golunda ellioti*. This study emphasizes the need for further entomological surveys in ST disease-endemic areas. Developing a morphological identification key for chigger mites in Sri Lanka is a top priority to facilitate field surveys.

Keywords: Chigger mites, Ectoparasites, Muridae, Scrub typhus

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Introduction

Scrub typhus (ST) is a mite-borne, acute febrile infectious disease in humans with the potential to cause severe or even fatal disease. It is also known as "tsutsugamushi disease" and "mite-borne typhus" (Kelly et al., 2009). The aetiological agent of ST infection is a gram-negative, pleomorphic, obligatory intracellular bacillus bacterium, termed *Orientia tsutsugamushi* (OT), formerly known as *Rickettsia tsutsugamushi* (Gurrant et al., 2013; Blanton et al., 2013; Guang et al., 2017). More than 20 antigenically distinct strains of OT have been reported, including the initially serologically distinguished prototypic strains Karp, Gilliam, and Kato. Scrub typhus is endemic to a region of 13,000,000 km^2 in the Asia-Pacific termed "The tsutsugamushi triangle," which extends from Afghanistan to China, Korea, the islands of the southwestern Pacific, and northern Australia. Recently, cases of ST have also been reported to occur in Chile (South America) and Cameroon (Africa), indicating the spread of the disease, caused by other species of Orientia, outside the traditional "tsutsugamushi triangle" (Ghorbani et al. 1997 Balcells, 2011). Over one million people are annually affected by ST, and over one billion people are at risk of acquiring the illness (Guang et al., 2013). Several countries, including China and South Korea, have recently experienced a rising incidence of ST (Wei et al., 2014; Kim et al., 2015). Thus, ST poses a considerable public health burden and threatens to widen its impact globally with the involvement of hitherto non-endemic regions.

Scrub typus is transmitted by the bite of a larval trombiculid mite (chigger) infected with OT. Mites are the primary reservoirs for OT. Among the different life cycle stages of trombiculid mites, only the larval stage is parasitic, with rodents acting as the primary hosts and humans as accidental hosts. The adult and nymphal stages of the mite are free living in the soil (Balcells et al., 2011). The geographic distribution of ST is determined by the habitation of the chigger mite vectors, which depend on many factors such as climatic factors, vegetation, and abundance of rodents. Tropical weather provides stable and ideal conditions for disease transmission, as mite activity is optimal in high temperatures and high humidity. Thus, the prediction of a global resurgence of ST owing to global warming, inflicting widespread human casualties, cannot be ignored (Young et al., 2020). In temperate countries, ST is seasonal due to the temporal activity of chiggers (Kelly et al., 2009).

The documented history of ST in Sri Lanka dates to the Second World War, with the first case reported in 1937 (Liyanapathirana and Thevanesam et al., 2011). The last few decades have shown a rising incidence. At present, an average of 1500 cases are notified annually, to the Epidemiology Unit, Ministry of Health, under the category of "Typhus" (Weekly Epidemiology Report, 2013). Since ST infection is documented with other types of rickettsioses in Sri Lanka, the recorded incidence and prevalence of ST disease need to be more accurate and specific. Although ST cases have been reported from all the districts in the country, the transmission of ST tends to be restricted to localities with mite activity. It is more prominent in the Western, Northwestern, Northern, and Southern provinces of the country (Liyanapathirana and Thevanesam 2011). In Sri Lanka, studies on trombiculid mites are limited. The first noted study on trombiculid mites was conducted by Jayewickrem and Niles (1946), who studied the role of trombiculid mites in the transmission of rural typhus. Wharton and Fuller (1952) reported the first list of species with five chigger mite species in Sri Lanka: *Blankaartia acuscutellaris* (Walch 1922), *Leptotrombidium akamushi* (Brumpt 1910), *Leptotrombidium deliense* (Walch 1922), *Ascoschoengastia indica* (Hirst 1915), and *Walchia* (*Walchia*) *turmalis* (Gater 1932). Several

studies on bats also record the presence of chigger mites in Sri Lanka (Brown *et al*., 2003). Recently, Ashani et al. (2022), provided an annotated checklist from 1946 to 2021 that comprised 15 species organized under nine genera from Sri Lanka.

Without licensed vaccines for scrub typhus, the only option available for control is mitigating the risk of contracting ST by avoiding vector contact (Peter et al., 2015). Thus, identifying disease vectors and their distribution are fundamental requirements in designing a control program for vector-borne infections. The lack of scientific knowledge on scrub typhus vector species and their distribution has been a significant obstacle to developing control strategies in the country. The limited studies in the country on chigger mites are grossly inadequate for decision-making. Hence, this study was conducted to update the knowledge on the species distribution and abundance of chigger mites in selected disease-endemic areas in Sri Lanka.

Materials and Method

Selection of study sites

Galle and Hambantota districts in the southern province and the district of Gampaha in the western province of Sri Lanka were selected for the field surveys. The selection of the Southern province was based on the annual incidence of typhus in 2017 and 2018, which was the province reporting the second highest number of cases. Gampaha district was selected based on the high case numbers reported to the Rickettsial Disease Diagnostic and Research Laboratory, Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka during last five years for disease confirmation. The selection of sampling sites was according to the patient distribution. Serologically confirmed cases of scrub typhus (a single high IFA IgG antibody titre of >1:128 to *O. tsutsugamushi* Karp, Gilliam, and Kato antigens) reported by the Rickettsial Disease Diagnostic and Research Laboratory, Faculty of Medicine, University of Kelaniya, Sri Lanka during 2017- 2019 was considered in selecting the study sites. These sites were visited, and GPS coordinates of the study sites were obtained using a handheld GPS monitor (Montana ®610 Garmin handheld GPS Receiver) and mapped using ArcGIS 10.

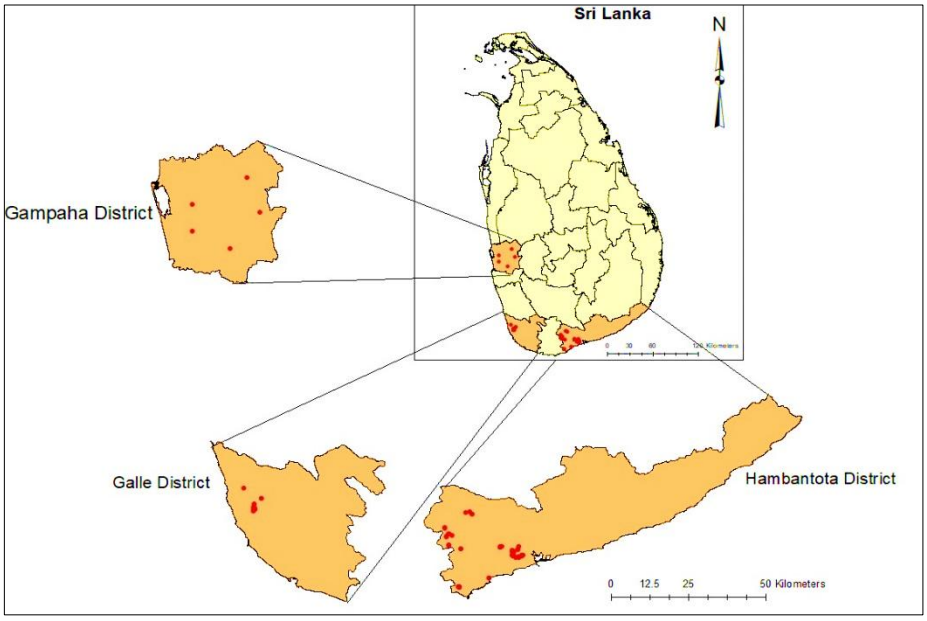

Figure 1 Distribution of field study sites

Sampling of chigger mites

Field sampling of mites was carried out at the selected study sites during 2019- 2020 by live capture of small rodents and small mammals, which are potential hosts of chigger mites. The trap locations were selected based on the possible exposure locations (PEL) of serologically confirmed cases/clusters of scrub typhus in each district. A mesh trap of 7.62 cm x 7.62 cm x 25.4 cm (W x H x L) in size was designed to capture rodents live using a bait (fried coconut). The traps were placed in peri domestic areas of recorded patient's residence and surrounding lands including agricultural lands namely paddy fields, cinnamon, and tea plantations just before sunset and retrieved the morning after. Rodents caught in traps were directed to a special anesthetizing chamber to administer ketamine (75mg/kg) /xylazine (10 mg/kg), and once anesthetized, the rodents were examined (especially in the ear lobes) for the presence of mites *in situ* (Figure 2). Rodents were observed and released to the captured habitat once they recovered from anesthesia.

Figure 2 The figure indicating (a): Trap used for capturing small rodents, (b): observing chiggers in the ear lobe of a field caught anaesthetized small rodent

Identification of chigger mites and morphometric characterization

Mites detected from the small rodents were removed carefully with a fine brush, collected (Figure 2), and washed individually with 10% phosphate-buffered saline (PBS) and slide-mounted in Hoyer's medium. Chiggers were identified at species level morphologically by visual inspection and morphometry using a camera-mounted light microscope (100x magnification). The morphological characteristics used here were based on original observations as previously reported in literature (Goff et al., 1982; Fernandes and Kulkarni, 2003). The publications of Wharton and Fuller (1952), Wormsley (1952), Goff (1982), and Fernandes and Kulkarni (2003)

were referred in the morphological identification process. Morphological characteristics were cross-checked with published literature and by examining reference specimens archived at the Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka and Laboratório de Coleções Zoológicas, Instituto Butantan, São Paulo, SP, Brazil. The slide-mounted mites were observed under 400x –1000x magnification of a light microscope (Olympus, N-300M [NOVEL]), and all measurements and images of mites were recorded (HDCE-X5, scope image 9.0, Bioimager software) for future reference.

Ethical approval

Ethical clearance for the study was obtained from the Ethics Review Committee (ERC) of the Institute of Biology, Sri Lanka (ERC IOBSL 196 5 2019).

Data analysis

Descriptive statistics (Percentage, numbers, and means) were used to characterize the abundance of different species collected from field surveillance and morphometric characterization of taxonomic features. The Shannon-Weiner species diversity index was calculated using the following formula $\mathbf{H'=-\sum(p_i\ ln(p_i))}$, where $\mathbf{p_i}$ is the proportion of each species ***i*** relative to the total number of species (Ortiz-Burgos, 2016).

Results

Screening of rodents for chiggers

A total of 422 mesh traps were placed at identified possible exposure locations in Galle (n=122), Hambantota (n=120) and Gampaha (n=178). A total of 58 small mammals were captured from Galle (n=19), Hambantota (n=7), and Gampaha (n=32). The captured small mammals belonged to species *Rattus rattus* (Black rat), *Rattus norvegicus* (Brown rat), *Tatera indica* (Indian gerbil), *Gollunda ellioti* (Indian bush rat) and *Suncus murinus* (Asian house shrew).

Chigger mites encountered from the field surveys.

A total of 379 chigger mites were characterized by morphological identification. Two species were identified as the genus *Leptotrombidium* Nagayo, Miyagawa, Mitamura, and Imamura 1916 and *Schoengastiella* Hirst 1915. *Leptotrombidium imphalum* Vercammen-Grandjean and Langston 1976 was the predominant species (75.46%; n=286), followed by *Schoengastiella punctata* Radford 1946 (8.44%; n=32). *Leptotrombidium imphalum* was found for the first time parasitizing the murids, *Rattus novergicus* (Berkenhout 1769), and *Tatera indica* (Hardwicke 1807) in new localities, recorded in Galle District, Southern Province, Sri Lanka. In addition, *S. punctata* was also recorded in a new locality (Gampaha District, Western Province) with a new host association, on *Golunda ellioti* Gray 1837. The list of species encountered with their numbers and percentage from the total collection is indicated in Table 1. The Shannon-Weiner Index (H) was 0.88, indicating the diversity of mite species in this sample. This index suggests that while *Leptotrombidium imphalum* is highly dominant, there is still some level of diversity.

Table 1. Chigger mite species encountered during the surveys.

Species	Number (n)	Percentage identified (%)	Proportion (p)	ln(p)	P * ln(p)
Leptotrombidium imphalum	286	75.46	0.75	-0.28	-0.21
Schoengastiella punctata	32	8.44	0.08	-2.47	-0.21
Leptotrombidium sp.	14	3.69	0.04	-3.30	-0.12
Microtrombicula sp.	19	5.01	0.05	-3.00	-0.15
Non trombiculid mites	28	7.39	0.07	-2.61	-0.19
Total	**379**	**100**			
Shannon-Weiner Index (H')					**H'= 0.88**

Morphometric characterization of chigger mites

A comparison of taxonomic characteristics between two species of chigger mites, *L. imphalum* and *S. punctata* is indicated in Table 2. These characteristics include measurements, setae arrangements, and leg segment details. For the taxonomic characteristics, the number of dorsal Setae of *L. imphalum* is 28-32, while *S. punctata* has 40 - 41. The number of ventral setae was higher in *S. punctata.* The scutum setae arrangement (*vi*/*ve*/*se*/*si*) of *L. imphalum* and *S. punctata* was 1/2/2/2 and 0/2/2/2/2, respectively. The average total length ranged from 250-600 µm in *L. imphalum* while it is 380 - 410 µm in *S. punctata.* These taxonomic characteristics provide important information for distinguishing between *L. imphalum* and *S. punctata*, aiding in identifying and classifying these chigger mite species. Captures of *L. imphalum* and *S. punctata* are included in Figures 3 and 4, respectively. Microscopic view of *Microtrombicula* sp. encountered in the study is illustrated in Figure 5.

Table 2. Morphometric characteristics of field-caught larvae of chigger mites *Leptotrombidium imphalum* and *Schoengastiella punctate*

Taxonomic character	*Leptotrombidium imphalum*	*Schoengastiella punctata*
Dorsal setae (number)	28-32	40-41
Ventral setae (number)	30-32	50-51
Scutum setae arrangement (*vi*/*ve*/*se*/*si*)	1/2/2/2	0/2/2/2/2
Total length	496.32 (250-600)	390.5 (380-410)

(AW) distance between anterolateral scutal setae	63 (50– 67.5)	43.75 (42.5 -47.5)
(PW) distance between posterolateral scutal setae	75.25 (62.5 – 77.5)	63.13 (62.5 – 65)
(SB) distance between sensilla	30 (27.5 – 35)	31.88 (30 – 32.5)
(ASB) distance from the level of sensillary bases to extreme anterior margin of scutum	25.25 (25-27.5)	25 (24-25)
(PSB) distance from the level of sensillary bases to extreme posterior margin of scutum	13.25 (10 -25)	83.12 (80 – 90)
(SD) length of scutum [ASB + PSB]	37.5 (32.5 – 52.5)	108.13 (105 – 117.5)
(P-PL) distance from the level of posterolateral scutal setae to extreme posterior margin of scutum	8.375 (5 -12.5)	68.75 (62.5 – 75)
(AP) distance from anterolateral to posterolateral scutal setae on one side	26.88 (25 – 35)	43.75 (42.5 – 45)
(vi) length of anteromedian seta of scutum	40 (45 – 52.5)	Ab
(Ve) external vertical setae	38.08(37.5 – 55)	27.5 (25- 30)
(Se) external scapular setae	50 (40 – 57.5)	37.5 (32.5 – 40)
(Si) internal scapular setae [trichobothria].	50 (37.5 – 62.5)	34.17(32.5 – 35)
Leg I; Length (µm) of leg segments and setae arrangement	250 (205 – 262.5)	210 (205 -212.5)
Leg II; Length (µm) of leg segments and setae arrangement	217.5 (187.5 – 215)	181.13 (175 – 187.5)
Leg III; Length (µm) of leg segments and setae arrangement	250 (212.5 – 262.5)	215.63 (200 – 237.5)
Number of Leg segments (leg I/ leg II/ leg III)	7,7,7	7,6,6
Coxal setae arrangement (I/II/III)	1/0/1	1/1/1

Data presented as mean with range within brackets in µm unless indicated otherwise.

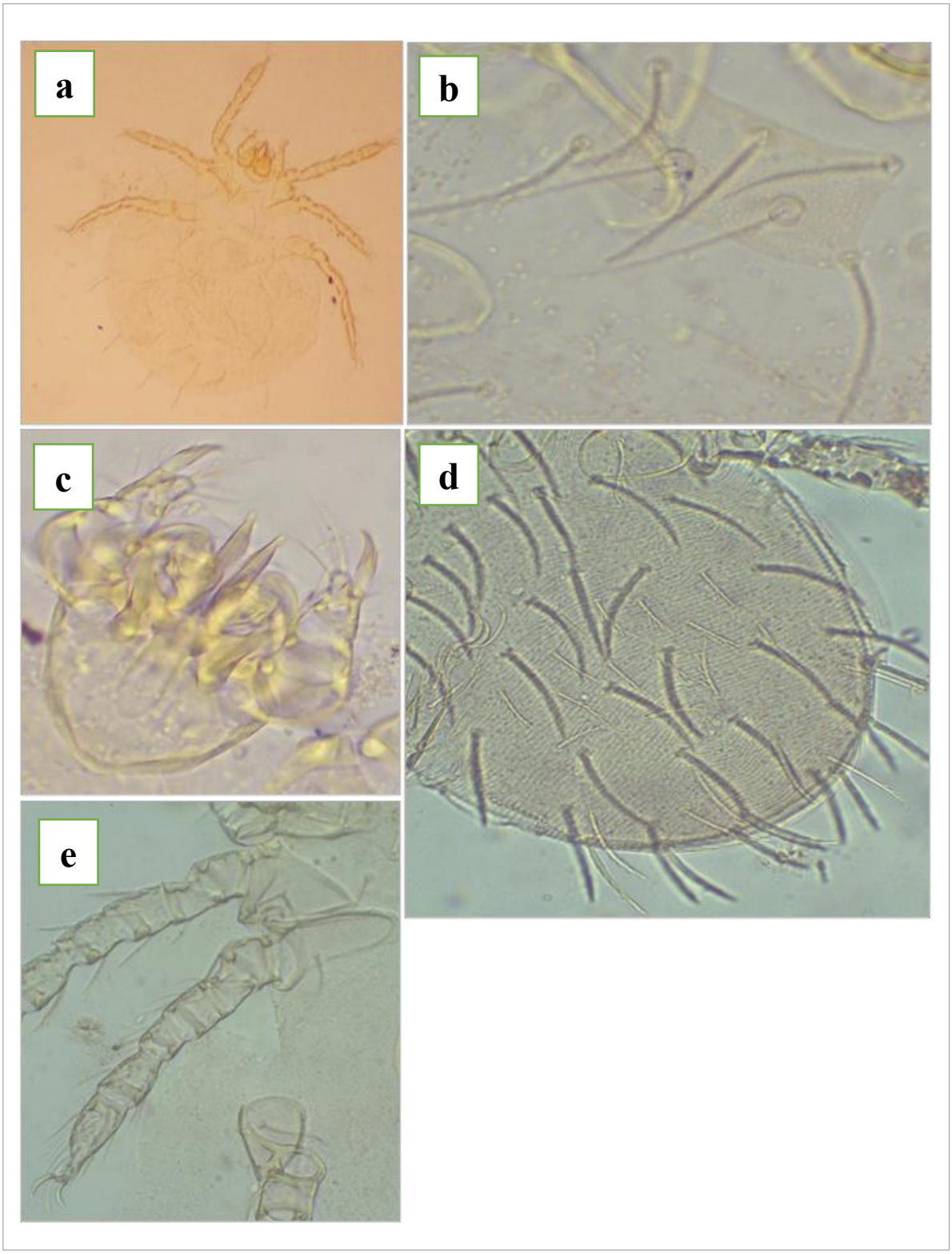

Figure 3 Microscopic view of *Leptotrombium imphalum* **(a);** whole mount -dorsal view (10 x10), **(b);** Scutum (10 x 60), **(c);** Gnathosoma (mouth parts) (10 x 60), **(d);** dorsal setae (10 x 60), **(e);** Leg I & II (10 x 60)

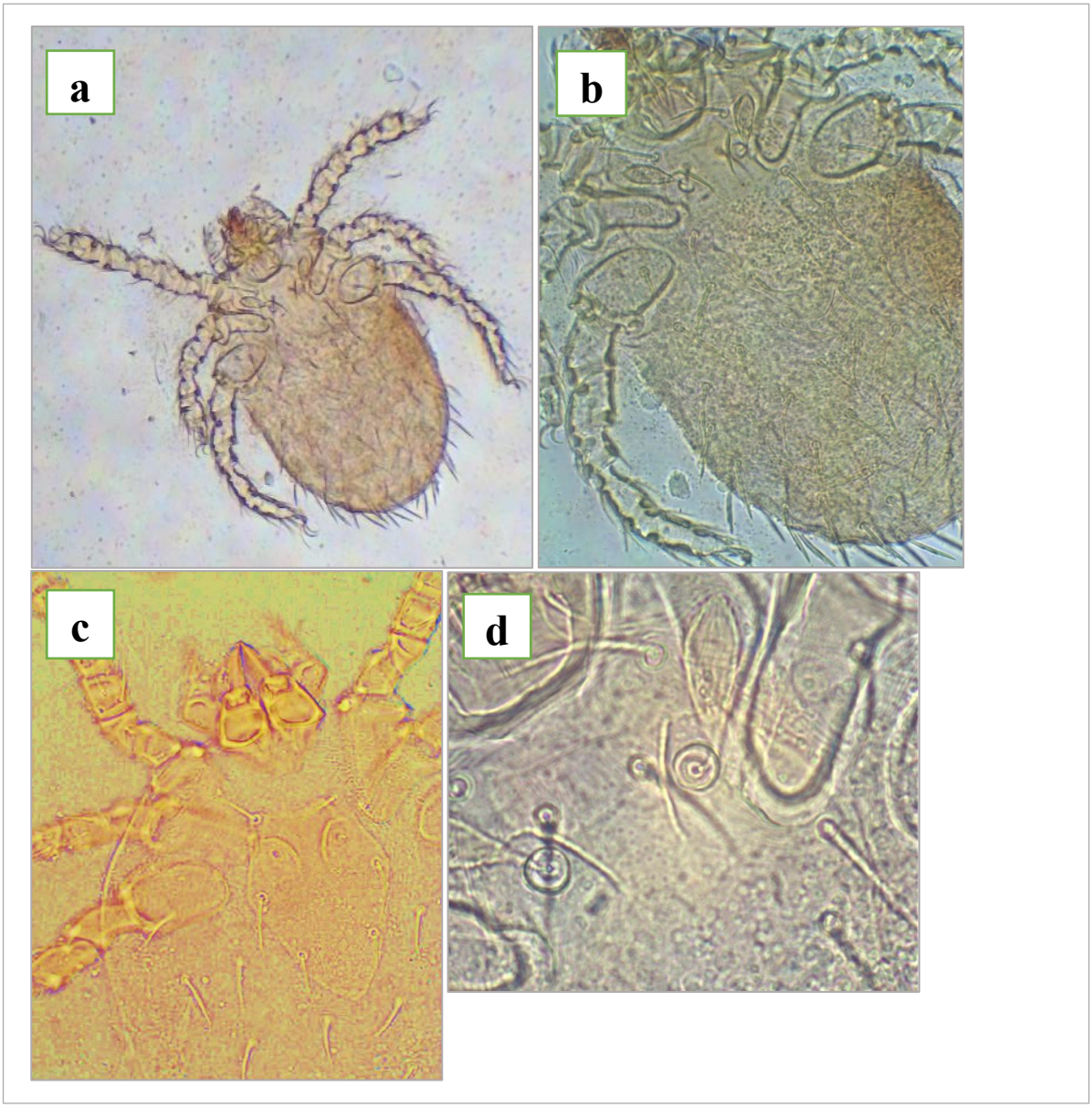

Figure 4 Microscopic view of *Schoengastiella punctata* **(a)** whole mount (10 x 20), **(b)** Whole mount (10 x 40), **(c)** Elongated scutum (10 x 60), **(d)** Scutum- sensila (10 x 100) Discussion

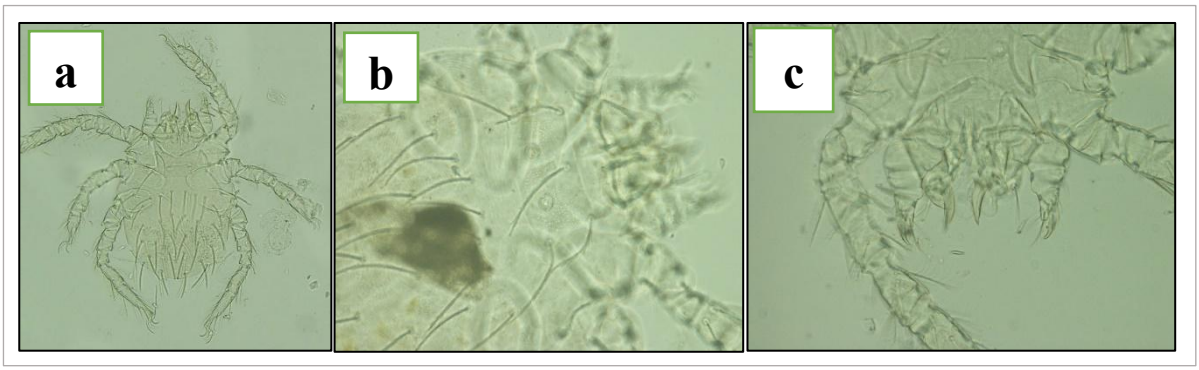

Figure 5. Microscopic view of *Microtrombicula* sp. **(a)** Whole mount (10 x 20)**, (b)** Scutum (10 x 60)**, (c)** Gnathosoma (10 x 100)

Discussion

In this study, three different genera: *Leptotrombidium*, *Schoengastiella*, and *Microtrombicula* of chigger mites were identified. Among these genera, *Leptotrombidium imphalum* was the most predominant species, comprising 72.59% of the collected specimens. This finding is consistent with previous studies that have identified *L. imphalum* as a common chigger species in Sri Lanka (Rajapakse et al., 2012) and commonly found in other countries (Traub, 1974), which has been recognized as a major vector of scrub typhus in many parts of Asia (Traub, 1974). Therefore, its high prevalence among the collected sample in the present study indicates its potential in transmitting scrub typhus in Sri Lanka.

The detection of *S. punctata* in a new locality (Gampaha District, Western Province) with a new host association (*Golunda ellioti*) is significant. This finding highlights the potential expansion of the distribution range of *S. punctata* and the broadening of its host range. Similar results have been reported in other studies conducted elsewhere, indicating the adaptability of chigger mites to different hosts and environments (Lane et al., 2006). The presence of *S. punctata* in a new locality emphasizes the need for continued surveillance and monitoring of chigger mite populations to track their distribution patterns and potential changes in host associations.

Another notable finding is the detection of *L. imphalum* parasitizing the murids *R. norvegicus* and *T. indica* in the district of Galle. This represents a new locality and host association for this chigger species. This discovery is important in expanding our knowledge of the host range of *L. imphalum*, which was previously primarily associated with small rodents such as *Rattus rattus* (Ruang-areerate *et al.,* 2015). The ability of chigger mites to utilize different host species could have implications for disease transmission dynamics and should be considered in future research and control strategies (Blackwell et al., 2002).

In this study, anesthetizing the captured animals at the study site was challenging as the animals resisted any handling probably due to the stress of capture. The procedure presented health hazards to the handler in case of a rodent bite, as some of these rodents may be infected with rabies and leptospirosis. To mitigate the issue, a low-cost special anesthetizing chamber was designed which could be directly connected to the commercially available trapping mesh cage. The trapped animal was encouraged to advance to this special chamber following, which the injectable anesthetic was administered to the trapped animal. This novel anesthetizing chamber allowed the researcher to anesthetize the captured animal with minimal physical restraint and maximum safety for the handler. This low-cost device injectable anesthetics may be administered to small animals without risk to the operator rendering it a valuable innovation for studies involving small mammals and rodents.

As in other field studies, this research also faced several limitations. The study collected 394 chiggers, which may not fully represent the diversity and distribution of chigger mites in Sri Lanka. A larger sample size would provide more comprehensive insights into the population dynamics and species composition of chigger mites in different regions of the country. The field sampling was conducted only in selected locations in three districts. These districts may only be fully representative of some of the country. Including additional sampling sites in different regions and ecological settings would provide a more comprehensive understanding of chigger

mite distribution and their host associations in Sri Lanka. Further, this study did not capture seasonal variations in chigger mite abundance and prevalence. Chigger mite populations and their interactions with rodent hosts can fluctuate throughout the year, and a longer sampling period would provide a more accurate representation of these dynamics. In addition, this study relied on the morphological identification of chigger mites based on visual inspection and morphometry. While this approach is commonly used, it may need to be improved in distinguishing closely related species and could lead to potential misidentification. Using additional molecular techniques, such as DNA barcoding, could enhance the accuracy of species identification. The study focused on capturing and examining rodents in areas where scrub typhus fever patients were located. However, detailed information on the abundance and diversity of rodent populations in these areas should have been provided. Understanding rodent population dynamics, habitat preferences, and their interaction with chigger mites is crucial for a comprehensive understanding of chigger mite ecology and disease transmission dynamics.

This study highlights the need for further entomological surveys in scrub typhus disease-endemic areas. The limited information available on chigger mites and their rodent hosts in Sri Lanka underscores the importance of expanding research efforts to understand better the ecology, behavior, and distribution of these vectors. Comprehensive entomological surveys can provide useful data for ST risk assessment to target control interventions. Additionally, the study emphasizes the necessity of developing a morphological identification key for chigger mites in Sri Lanka. Such a key would enable accurate and efficient species identification during field surveys, facilitating data collection on species distribution and abundance.

Conclusions

In conclusion, this study expands our knowledge of chigger mite species composition and associated hosts in selected districts in Sri Lanka. Identifying new host associations and localities for certain chigger species highlights the dynamic nature of chigger mite populations. The findings emphasize the need for continued entomological surveys and the development of identification tools to support effective surveillance and control measures for ST and other chigger-borne diseases in Sri Lanka.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Supplementary material

Different small rodents (hosts) captured in the field surveys, **(a);** *Gollunda ellioti* (Indian bush rat), **(b);** *Suncus murinus* (Asian house shrew), **(c);** *Rattus norvegicus* (Brown rat), **(d);** *Rattus rattus* (Black rat), **(e);** *Tatera indica* (Indian gerbil)

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