Trends in Parasitology

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Review

The chigger microbiome: big questions in a tiny world

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'Chiggers' (trombiculid mite larvae) are best known as vectors of rickettsial pathogens, *Orientia* spp., which cause a zoonosis, scrub typhus. However, several other pathogens (e.g., Hantaan orthohantavirus, Dabie bandavirus, *Anaplasma* spp., *Bartonella* spp., *Borrelia* spp., and *Rickettsia* spp.) and bacterial symbionts (e.g., *Cardinium*, *Rickettsiella*, and *Wolbachia*) are being reported from chiggers with increasing frequency. Here, we explore the surprisingly diverse chigger microbiota and potential interactions within this microcosm. Key conclusions include a possible role for chiggers as vectors of viral diseases; the dominance in some chigger populations of unidentified symbionts in several bacterial families; and increasing evidence for vertical transmission of potential pathogens and symbiotic bacteria in chiggers, suggesting intimate interactions and not simply incidental acquisition of bacteria from the environment or host.

Highlights

In addition to agents of scrub typhus (*Orientia* spp.), several other bacterial and viral pathogens have been recorded from chiggers, some of which appear to be vertically transmitted.

A number of arthropod symbionts have also been reported from trombiculid mites, and there is strong evidence for vertical transmission for approximately half of these taxa.

Very few unbiased microbiome studies of chiggers have been conducted to date and have taken very different approaches (i.e., analysis of a mite colony or wild chiggers, whether fed or questing), rendering meaningful comparisons difficult; moreover, three of four studies were undertaken in the same country (Thailand).

The vast majority of studies on pathogens or arthropod symbionts in chiggers have focused on bacteria, with no unbiased virome or mycobiome analyses performed to date.

Medical and veterinary importance of chiggers

The larval stage of trombiculid mites (Acari: Trombiculidae), known colloquially as **chiggers** (see Glossary), are the sole confirmed vectors of intracellular bacterial pathogens of the genus *Orientia*, the causative agents of scrub typhus. Chiggers parasitize mainly wild vertebrate hosts, such as small mammals, reptiles, and birds, whereas humans can be incidental hosts. *Orientia* spp. are closely related to *Rickettsia* spp., a better-known genus that includes the arthropod-borne pathogens responsible for epidemic typhus, endemic typhus, and spotted fevers. Scrub typhus is a zoonotic febrile illness with a wide distribution in the Asia-Pacific Region, where the disease is caused by *Orientia tsutsugamushi*. If not diagnosed and treated promptly, scrub typhus can lead to complications including pneumonitis, myocarditis, and encephalitis, with a median fatality rate of 6% [1,2]. Since the early 21st century, endemic scrub typhus has become recognised outside the Asia-Pacific Region, leading to the identification of ***Candidatus*** Orientia chuto from the Middle East and East Africa [3–5] and *Candidatus* Orientia chiloensis from South America [6]. Molecular data from wildlife reservoirs and chiggers, as well as human serological surveys, indicate that *Orientia* is circulating in other parts of the world, including Africa, where suspected human cases have been recorded [7].

Only chiggers of the genus *Leptotrombidium* are known to transmit *O. tsutsugamushi* to humans, while in Chile, *Herpetacarus antarctica* has recently been identified as a vector of scrub typhus caused by *Candidatus* O. chiloensis [8,9]. For *Candidatus* O. chuto, the vector(s) transmitting human disease have not been confirmed, although the organism has been detected in rodent-derived chigger pools consisting of *Neotrombicula* spp. and *Microtrombicula* spp. in Kenya [4]. However, approximately 15 chigger genera in total have been found to be infected with *Orientia* spp. in the wild (Table 1) and probably serve as intrazoonotic vectors that facilitate maintenance of *Orientia* spp. in wildlife [2]. It should be noted that chiggers can also be a serious biting nuisance in their own right, both in scrub typhus-endemic and non-endemic regions, due to pruritic dermatitis (trombiculiasis or 'scrub itch') resulting from exposure to mite allergens during feeding [10–12]. In

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Table 1. List of potential microbial pathogens of vertebrates detected in chiggers by targeted surveys

Microbial taxa	Pathogen species	Chigger species[a]	Refs
Virus (Bunyavirales)	Bayou orthohantavirus (Hantavirus)	Unidentified chiggers	[27]
	Hantaan orthohantavirus (Hantavirus)	*Leptotrombidium scutellare*	[24–26]
	Dabie bandavirus (severe fever with thrombocytopenia syndrome virus)	*L. scutellare* and *Leptotrombidium deliense*	[31,32]
Bacteria (Spirochaetia)	*Borrelia burgdorferi* sensu lato	*Neotrombicula autumnalis*	[33]
	B. garinii	*Neotrombicula* spp.	[34]
	B. valaisiana	*Neotrombicula* spp.	[34]
	Borrelia spp.	*L. deliense* and unidentified pooled chiggers*	[50,51]
Bacteria (Alpha-proteobacteria)	*Anaplasma phagocytophilum*	*N. autumnalis*	[35]
	Bartonella tamiae	*Blankaartia* sp., *Leptotrombidium* sp. and *Schoengastia* sp.	[36]
	Bartonella spp.	Unidentified pooled chiggers*	[37]
	Orientia tsutsugamushi	At least 35 chigger species, e.g., *Ascoshoengastia indica, Blankaartia acuscutellaris, Eushoengastia koreaensis, Eutrombicula wichmanni, Gahrliepia saduski, G. xiaowoi, Guntheria cassiope, Helenicula miyagawai, Leptotrombidium akamushi, L. arenicola, L. arvinum, L. bodense, L. deliense, L. fletcheri, L. fuji, L. gaohuensis, L. imphalum* (or synonym *L. chiangraiensis*), *L. intermedium, L. keukenshrijveri, L. kitasatoi, L. orientale, L. pallidum, L. palpale, L. pavlovskyi, L. peniculatum, L. scutellare, L. vivericola, Microtrombicula chamlongi, Neotrombicula japonica, Odontacarus* sp., *Shoengastiella ligula, Schoutedenichia* sp., *Trombiculindus variaculum, Walchia kritochaeta, W. pacifica*	[63,64,76–86]
	Candidatus Orientia chuto	Unidentified pooled chiggers*	[4]
	Candidatus Orientia chiloensis	*Herpetacarus antarctica* and *Herpetacarus eloisae*	[8,9]
	Rickettsia aeschilimannii	*L. scutellare*	[48]
	Rickettsia akari	*L. scutellare* and unidentified pooled chiggers*	[38,48]
	Rickettsia australis	*L. scutellare* and unidentified pooled chiggers*	[38,48]
	Rickettsia conorii	*Eutrombicula* sp., *Leptotrombidium peromysci* and unidentified pooled chiggers*	[38,45]

Glossary

***Candidatus*:** a prefix used for a prokaryotic species that has been characterized (at least in part) using molecular data but the name of which is not considered validly published, either because it cannot be cultivated in the laboratory or existing cultures have not been deposited in public culture collections.

Chiggers: the common colloquial term used in parts of Asia for the tiny parasitic larval stages of mites in the family Trombiculidae, also known as 'redbugs' (North America), 'harvest mites' (Europe), 'scrub itch mite' (Australia), and 'tsutsugamushi' or 'kedani' (Japan).

Cytoplasmic incompatibility: a form of reproductive parasitism in which a vertically transmitted bacterium modifies sperm in infected male arthropods, such that after mating with an uninfected female (or a female infected with an incompatible bacterial strain), the resultant embryos die.

Gamasid mites: a group of mites of the order Mesostigmata; they live either as predators in the soil, feeding on smaller arthropods and nematodes, or as parasites of invertebrate or vertebrate animals, some of these parasites having a tick-like lifestyle.

Microbial β-diversity: a measure of the similarity or dissimilarity of two microbial communities/compositions.

Operational taxonomic units (OTUs): in microbiology, clusters grouped by DNA sequence similarity for a specific taxonomic marker gene, most commonly 16S rDNA, which can include both well-characterized organisms and uncultivated, potentially novel, species.

Stylostome: a tube-like cavity formed by chigger salivary secretions solidifying in the host epidermis or dermis; it is used by chiggers to feed on host cells and exudate.

Terpenoids: naturally occurring organic chemicals derived from isoprene and its polymers (terpenes), which are synthesized mainly by plants, bacteria, and fungi. In arthropods, terpenoids can be perceived as attractants to a food source, repellents that deter ingestion, or as pheromones that trigger aggregation and mating behaviour.

Table 1. (continued)

Microbial taxa	Pathogen species	Chigger species[a]	Refs
	Rickettsia felis	*Blankaartia sinnamaryi, Eutrombicula* sp., *Leptotrombidium peromysci* and *L. scutellare*	[45,46,48]
	Rickettsia helvetica	*Hirsutiella zachvatkini* and *Kepkatrombicula storkani*	[40]
	Rickettsia japonica	Unidentified pooled chiggers*	[38]
	Rickettsia monacensis	*Hirsutiella zachvatkini*	[40]
	Rickettsia typhi	*Eutrombicula* sp., *L. peromysci* and unidentified pooled chiggers*	[38,45]
	Rickettsia sp. TwKM02	*L. deliense* and unidentified pooled chiggers*	[38,39,44]
	Rickettsia sp. TwKM03	*L. deliense* and unidentified pooled chiggers*	[39]
	Rickettsia sp. MB74-1	Unidentified pooled chiggers*	[44]
	Rickettsia sp. Cf15	Unidentified pooled chiggers*	[38]
	Rickettsia spp.	*Cheladonta costulata, Eutrombicula* sp., *Hirsutiella zachvatkini, L. peromysci, L. scutellare, N. autumnalis, Neotrombicula vulgaris*, unidentified pooled chiggers*	[38,40,43,45,47,48]
	Candidatus Rickettsia colombianensi	*Eutrombicula tinami*, *B. sinnamaryi*, *Herpetacarus hertigi, Quadraseta trapezoides*, and *Trombewingia bakeri*	[42,47]
	Candidatus Rickettsia leptotrombidium	*L. scutellare*	[41]

[a]An asterisk (*) indicates that the bacterial pathogen was detected in pooled chigger samples of unidentified species.

addition to humans, dogs, cats, horses, and domestic ruminants can be affected severely by trombiculiasis worldwide [13–15].

Among human disease vectors, chiggers are unusual for two key reasons. First, they do not feed on blood. Chiggers use a unique mode of feeding in which a straw-like structure called the **stylostome** (Box 1) is produced by secreted substances only after the chigger has attached to the host (Figure 1B). Digestive enzymes are pumped via the stylostome into the skin, and the chigger ingests tissue fluid and liquified cells [16] (Figure 1B). Second, in most circumstances, chiggers are thought to feed only once and then drop off the host to moult via three nymphal stages (i.e., protonymph, deutonymph, and tritonymph) into adults (Figure 1A). The post-larval stages are free-living predators in soil, feeding primarily on the eggs of other arthropods. Thus, a pathogen acquired by a chigger during feeding on an infected host (unless perhaps feeding is interrupted) could not be transmitted to a new host without transstadial transfer through the free-living stages, followed by vertical inheritance via the female germline into the next generation (transovarial transmission). Laboratory studies using *O. tsutsugamushi*-infected rodents suggest that, while acquisition of the pathogen by chiggers is common during feeding, as is transstadial transfer, subsequent vertical transmission is very rare [17]. However, since individual hosts can be parasitized by hundreds or even thousands of chiggers [18,19], rare events could be very important epidemiologically.

Box 1. Chigger feeding via the stylostome and bacterial acquisition

Chigger feeding behaviour is often misunderstood as it is fundamentally different to that of other parasitic mites or ticks – they are neither blood-sucking nor burrowing mites. First, chiggers use strong blade-like mouthparts, chelicerae, to pierce the host's skin. During feeding, extra-oral digestion is a key process that is dependent on the formation of a straw-like cavity called the 'stylostome'. This develops from chigger saliva, leading to the construction of a complex glycoprotein structure extending from the stratum corneum into the host's epidermis or dermis [16]. Powerful enzymes from the salivary gland are released into the wound, and the larvae are then able to feed on the digested tissue, lymphoid, and cellular components by pumping the lysate into their body. Salivary glands of newly hatched chiggers already contain secretory granule enzymes, suggesting the potential ability to feed on a host very soon after hatching [72].

The structure, depth, and width of stylostome formation is different between chigger species [16,73,74], but the potential impact of this variation on the probability of acquiring pathogens during feeding is not known [52]. However, bacterial taxa exhibiting a high affinity for the external layers of connective tissue, including skin, would seem more likely to be acquired or transmitted during feeding activity than those residing in peripheral blood or deeper tissues. In addition, the depth of stylostome penetration into the skin and ability to disseminate digestive enzymes from saliva into a wider area of host tissue may influence the degree of inflammation and the dissemination of pathogens around the bite site [73,75].

Can chiggers transmit viruses?

Hantaan orthohantavirus is the causative agent of Korean haemorrhagic fever in the Far East; that is, north-eastern China, Korea, and eastern Russia. Similar to scrub typhus, Hantaan orthohantavirus is a zoonotic infection maintained in rodents without causing mortality in its natural host (principally, the striped field mouse, *Apodemus agrarius*) [20,21]. However, unlike *Orientia* spp., Hantaan orthohantavirus is considered to be transmitted directly between rodents or to humans without the need for an arthropod vector, either via inhalation of aerosolized excreta or by biting [21–23].

This paradigm of direct transmission has been challenged by research from Chinese scientists over the past 60 years; they have implicated chiggers (as well as **gamasid mites**) in the epidemiology of this infection [24–26] (Table 1). Key findings were that field-collected questing chiggers or adult mites (mainly *Leptotrombidium scutellare*, one of the principal scrub typhus vectors in China) could be found naturally infected with the virus, and chiggers could transmit it to naïve rodents in the laboratory. Moreover, transstadial and vertical transmission of the virus was demonstrated [24,25], indicating that chigger infection is not just a transient by-product of feeding on infected hosts. Corroborating data were obtained from a field site in Texas, where RNA of a different hantavirus (Bayou) was detected in chiggers feeding on hantavirus-negative rodent hosts, as well as a free-living trombiculid in the predatory stage of the life cycle collected from soil [27]. These laboratory and field studies are further supported by epidemiological data indicating that meteorological factors associated with mite abundance (e.g., relative humidity) after the rodent breeding season were predictive of peak Hantaan orthohantavirus transmission to humans in Qingdao, China [28]. Finally, a study in South Korean soldiers showed that the use of insecticides or insect repellent, and sleeping in barracks rather than on ground outside, were the most protective factors against acquisition of Korean haemorrhagic fever [29]. Despite this evidence of the involvement of chiggers and other mites in hantavirus transmission, the established paradigm of direct transmission from rodents to humans is still considered the mainstream consensus [30].

Transmission of the tick-borne Dabie bandavirus by chiggers is also suspected according to a report in the Chinese literature [31]. Genomic RNA of this human pathogen, also known as severe fever with thrombocytopenia syndrome virus (SFTSV), was detected in *L. scutellare* and a gamasid mite (*Laelaps echidninus*) obtained from field mice trapped in Jiangsu Province, China. Recent evidence from another small mammal field survey in rural China reported a low SFTSV infection rate of 0.2% in *Leptotrombidium deliense* collected from the rodent host, whereas a high prevalence of the virus (>30%) in the same rodent population was revealed [32]. To date, these are the only studies reporting detection of SFTSV genetic material in chiggers.

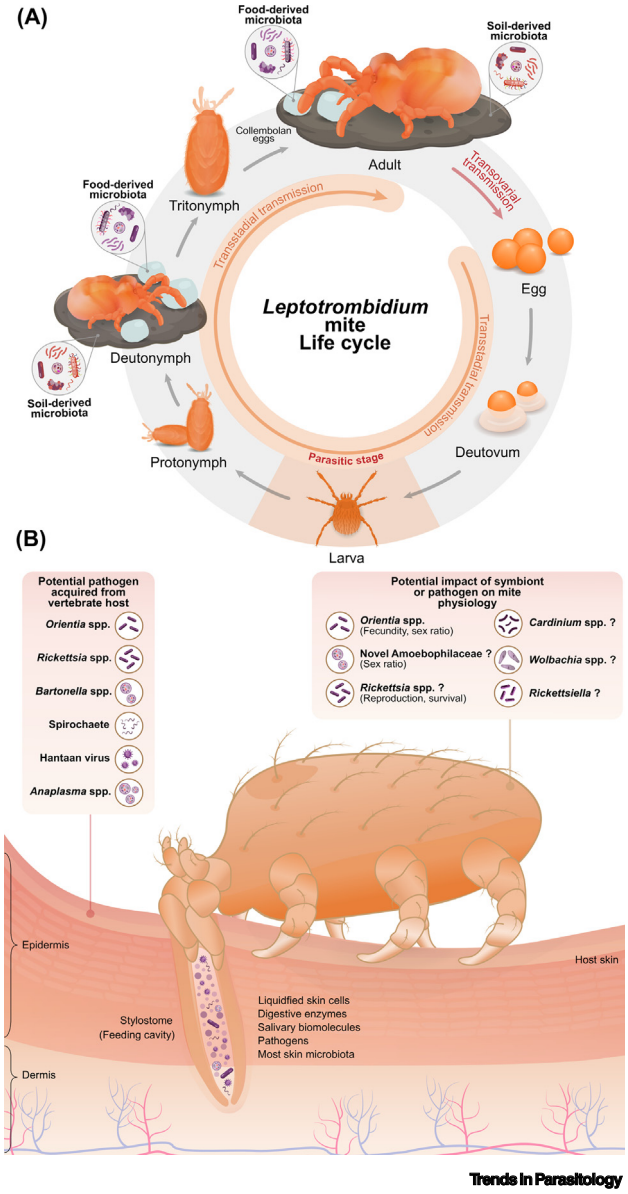

Figure 1. Potential sources of microbiota in trombiculid mites. (A) The life cycle of trombiculid mites (*Leptotrombidium* spp. as an example). Larvae or 'chiggers' are the only parasitic stage, whereas the active post-larval stages (deutonymphs and adults) are edaphic predators, feeding primarily on the eggs of other arthropods (e.g., collembolans). The protonymph and tritonymph are quiescent stages. Microorganisms acquired during larval feeding can transiently infect the chigger or become established long-term through transstadial transfer and vertical transmission via the female germline. For the scrub typhus pathogen, *Orientia*, transmission into the F1 generation from infection acquired as larvae is very rare [17], but the frequency of these events for other acquired pathogens is unknown. Other potential sources of microbiota contributing to the trombiculid microbiome are soil and food ingested by the free-living stages. However, while the *Leptotrombidium* genome contains laterally transferred genes from soil microbiota [12], implying an intimate relationship, studies on a laboratory colony suggest limited acquisition of bacteria from the diet [49]. (B) Mode of feeding of chiggers. The straw-like feeding cavity or 'stylostome' (Box 1) does not penetrate capillaries but enables intake of host cells that have been digested by secreted mite enzymes. Skin microbiota as well as bacterial and viral pathogens could be acquired from the host during feeding as indicated (box on left), although an alternative source of pathogens might involve transfer between cofeeding chiggers [2]. Vertically transmitted bacterial symbionts recorded from chiggers (box on right) could have effects on mite physiology and reproduction or might interfere with pathogen transmission.

However, there is a lack of empirical evidence demonstrating that the virus can be vectored by chiggers and/or other mites, as ectoparasites feeding on potentially viraemic hosts could simply contain viral remnants in the gut or live virus that cannot be transmitted further.

The presence of non-*Orientia* bacterial pathogens in chiggers

Several bacterial pathogens (or bacterial genera with pathogenic potential) other than *Orientia* spp. have been detected in chiggers (Table 1). However, only one study has sought to demonstrate transmission of non-*Orientia* pathogens in the laboratory, which involved members of the *Borrelia burgdorferi* sensu lato complex (agents of Lyme borreliosis). Thus, infection of harvest mites (*Neotrombicula autumnalis*) by *Borrelia garinii* was observed after feeding on experimentally

infected mice, although there was no attempt to demonstrate subsequent transstadial transfer, vertical transmission from adult females, and infection of naïve rodents by the first filial generation (F1) larvae. The same study also reported the detection of *B. burgdorferi s. l.* DNA in an *N. autumnalis* larva collected from a shrew in Germany, while a nymph reared in the laboratory appeared to have acquired *Borrelia valaisiana* in the wild, possibly via vertical transmission [33]. In a subsequent study from the Czech Republic, *Neotrombicula* spp. were collected from wild birds, and one pool of five larvae was positive for both *B. garinii* and *B. valaisiana*. Since the bird host (the Eurasian blackcap, *Sylvia atricapilla*) was not known to be an important reservoir of *B. burgdorferi s. l.*, the authors speculated that the larvae had acquired *Borrelia* spp. infections vertically before feeding [34]. Whereas no *Anaplasma phagocytophilum* (agent of human granulocytic anaplasmosis and tick-borne fever in ruminants) infections were detected in *Neotrombicula* spp. in the bird study, an earlier survey from Spain detected *A. phagocytophilum* DNA in questing *N. autumnalis*, indicating vertical transmission [35]. These findings for both *B. burgdorferi s. l.* and *A. phagocytophilum* in chiggers are surprising, as the principal vectors of these pathogens are hard ticks in which vertical transmission does not occur.

At least two other alpha-proteobacteria with pathogenic potential, *Bartonella* spp. and *Rickettsia* spp., have been detected in numerous surveys of feeding chiggers (Table 1). The DNA of *Bartonella tamiae*, a pathogen causing human bartonellosis in Thailand, was detected in several genera of chiggers (*Blankaartia, Leptotrombidium*, and *Schoengastia*) collected from rodents in both northern and southern regions of the country [36]. Furthermore, nucleotide sequences from chiggers closely matched those from human clinical isolates, although no further studies to evaluate chigger-mediated transmission to naïve rodents or vertical transmission in unfed larvae have been performed. In Vietnam, *Bartonella* spp. were detected in pooled chigger samples from the Mekong Delta region. The rate of *Bartonella* infection in the chiggers collected on *Bartonella*-infected rats was higher than for chiggers from uninfected rats, although these findings are difficult to interpret without studies on potential transmission of *Bartonella* spp. by chiggers using laboratory animals [37].

Rickettsia spp. constitute the final group of potential pathogens recorded from chiggers (Table 1). Indeed, *Rickettsia* spp. may be more widespread in chiggers globally than *Orientia* spp. To date, *Rickettsia* spp. DNA has been detected from fed chiggers collected from small mammals in Brazil, the USA, Slovakia, South Korea, Thailand, Vietnam, mainland China, and Taiwan [38–45], as well as in chiggers parasitizing birds in Brazil [46,47]. Importantly, *Rickettsia* spp. DNA (as well as that from two other endosymbionts: *Wolbachia* and *Rickettsiella*) has also been amplified from questing *L. scutellare* in Japan, demonstrating potential vertical transmission [48]. Genetic analyses indicate that several different rickettsial strains are present in chiggers, with most falling in the Transitional group. This clade includes both important pathogens such as *Rickettsia australis* (agent of Queensland tick typhus) and *Rickettsia akari* (agent of rickettsialpox, which is transmitted by gamasid mites), as well as endosymbionts of ticks that have not been associated with human infections (e.g., *Rickettsia hoogstraalii*). Thus, it is currently unclear if *Rickettsia* spp. in chiggers are arthropod-restricted symbionts or vertebrate pathogens, or a mixture of both. However, '*Rickettsia* sp. clone MB74-1' and '*Rickettsia* sp. TwKM02' exhibit a degree of specificity for chiggers, as according to a Taiwanese study, they are rarely present in the rodent host or coinfesting ectoparasites such as fleas and ticks [44]. Molecular detection of these strains was achieved using different gene targets and they may in fact be the same species. Similar sequences have been reported from chiggers in South Korea [38].

The chigger microbiome and its origin

Only four studies to date have explored the chigger microbiome using an unbiased approach involving 16S rRNA amplicon sequencing. The first such study was performed with a laboratory

colony of *Leptotrombidium imphalum*, an important vector of *O. tsutsugamushi* in South-East Asia [49]. Both *Orientia*-infected and uninfected mites were examined in multiple stages of the life cycle (larvae, deutonymphs, and adults of both sexes). In infected mites, especially adult females, *O. tsutsugamushi* dominated the microbiome alongside a novel member of the family Amoebophilaceae. Together, these two **operational taxonomic units** (**OTUs**) accounted for >98% of reads in this stage and their co-occurrence was statistically significant. In uninfected mites, the microbiome was more diverse and the genera *Luteimonas*, *Ralstonia*, *Propionibacterium*, *Mycobacterium*, *Streptococcus, Staphylococcus, Corynebacterium*, and *Streptomyces* were the most abundant.

The first analysis of the microbiome of wild chigger specimens was conducted in northern Thailand, with a focus on detection of arthropod-borne human pathogens in ectoparasites retrieved from small mammals [50]. In chiggers (species of which were not reported in this study), most bacterial reads belonged to Gram-positive genera such as *Corynebacterium, Bacillus*, and *Staphylococcus*. Corroborating the targeted detection of potential pathogens discussed in the preceding text, some chiggers were found to harbour *Bartonella* and *Borrelia* (in addition to *Orientia*); while symbionts of the genera *Cardinium*, *Coxiella*, and *Francisella* were also detected. A second study involving the analysis of the chigger microbiome from Thai small mammals took a systematic approach across 11 provinces along habitat gradients, comparing individual specimens with pools for eight widespread chigger species from the genera *Leptotrombidium*, *Ascoschoengastia*, *Walchia*, *Helenicula*, *Blankaartia*, and *Schoengastiella* [51]. In accordance with the previous 16S rRNA amplicon sequencing studies, *Corynebacterium, Mycobacterium, Staphylococcus*, and *Streptomyces* were among the dominant OTUs. However, *Orientia* (detected only in *L. deliense* in this study) did not dominate the microbiome when present, and there was no evidence for the novel Amoebophilaceae OTU. While *Borrelia* was common in pooled chigger samples, especially for *L. deliense*, neither *Rickettsia* nor *Bartonella* was detected. The most abundant potential symbiont was a member of the Neisseriaceae, although *Cardinium*, *Rickettsiella*, and *Wolbachia* were also observed at lower rates. Surprisingly, a thermophilic OTU (*Geobacillus*) occurred at a prevalence of >80% in individual samples. Key drivers for **microbial β-diversity** were found to be chigger species and geographic origin, but not finer-scale habitat type.

The most recent unbiased analysis of the microbiome of a trombiculid was conducted on *Leptotrombidium scutellare* larvae in Japan. Although no evidence of *Orientia* was found in these chiggers, three other intracellular symbionts were revealed (*Rickettsia*, *Rickettsiella*, and *Wolbachia*), as well as an unknown member of the Rickettsiales [48]. Several rickettsial OTUs were similar to pathogenic rickettsial species reported from human infections: *Rickettsia aeschlimannii, R. akari, R. australis*, and *Rickettsia felis*. The *Rickettsiella* and *Wolbachia* sequences were classified phylogenetically into distinct clades from previously known strains, suggesting they are novel taxa that might have evolved independently in the trombiculid mites. As the larvae studied were unfed, these are particularly interesting data, as they provide evidence of vertical transmission of multiple symbionts from the parents to eggs and subsequently to the unengorged larvae.

As a result of their importance as vectors of scrub typhus, most targeted surveys and unbiased microbiome profiling in chiggers have been focused on *Leptotrombidium* spp., although the European harvest mite (*Neotrombicula autumnalis*) has also attracted considerable attention due to its propensity to feed on humans and companion animals (Table 1). However, a recent checklist [52] identified 100 chigger species from 28 genera for which data on associations with potentially pathogenic bacteria are available. In total, 31 bacterial taxa in eight families

(Anaplasmataceae, Bartonellaceae, Borreliaceae, Coxiellaceae, Francisellaceae, Leptospiraceae, Mycobacteriaceae, and Rickettsiaceae) were reported from trombiculid mites worldwide, which includes findings from the microbiome studies discussed in the preceding text. Many of these chigger species are unlikely to feed on humans, although they could potentially transmit pathogens to nonhuman vertebrates, including domesticated species, which can suffer from heavy chigger infestations [13–15].

A key question is the source of the microbiome of chiggers for those bacterial species that are not vertically transmitted. For the larvae, environmental sources (primarily soil) and the host (including skin and fur, as well as ingested bacteria) would be expected to be the main horizontal routes of microbiome acquisition (Figure 1B), whereas for the free-living stages, soil and prey items are likely origins (Figure 1A). However, in laboratory colonies of *L. imphalum*, there was minimal overlap between the diverse microbiome of collembolan eggs and that of the nymphs and adult mites that fed on them [49]. Furthermore, analysis of soil samples from South-East Asia suggested that the microbiome of fed chiggers is not consistent with simple retention of soil particles on the cuticular surface [51]. Of the four unbiased microbiome studies, only one used a dedicated surface sterilization step prior to DNA extraction from chiggers and 16S rRNA amplicon sequencing [48], although the effectiveness of this was unclear since reads for OTUs representing anything other than obligate intracellular bacteria were filtered out as a first step in the analysis. Sterilization usually involves the use of ethanol (incidentally used as a chigger fixative in the three other microbiome studies) or dilute bleach (sodium hypochlorite) and is frequently applied in microbiome analyses of ticks and fleas [53,54]. Whether surface sterilization is effective or necessary to improve the accuracy of arthropod microbiome studies remains controversial. An investigation of insect storage and handling methods for microbiome analysis revealed little impact of surface sterilization [55], whereas a similar study on adult ticks demonstrated that bleach removed bacterial DNA from the cuticle whereas ethanol did not [56]. However, because chigger specimens are particularly small and prone to damage during handling, application of a powerful denaturant such as bleach might risk a significant reduction in DNA yield. Irrespective of attempts to remove surface-associated bacterial DNA, it is important that negative controls are sequenced in low-biomass microbiome studies, such as those involving small arthropods, in order to account for bacterial DNA contamination from laboratory equipment and/or reagents. One of the chigger microbiome studies incorporated three different types of background controls into their analysis and also excluded the possibility that the high proportion of reads from a thermophilic bacterium (*Geobacillus* spp.) in chiggers originated from the laboratory water bath [51].

It should be noted that bacteria on the arthropod cuticle may not simply represent incidental contaminants, as ectosymbionts conferring important benefits to their hosts have been reported from several arthropod groups [57,58], while certain pathogens, including some *Rickettsia* spp., can be transmitted environmentally via vector faeces [54,59]. One weakness of the chigger microbiome studies conducted to date is that data on the precise location of microbiota in or on the mites are lacking, and neither were bacterial species accurately quantified within the microbiome. Moreover, most chigger microbiome analyses did not obtain sequencing data from additional loci to characterize bacterial species or strains, although one Thai study did confirm that *Coxiella* spp. in chiggers was a symbiotic species, not the Q-fever agent, *C. burnetii* [50].

Potential effects of the microbiome on its host

Further evidence for long-term microbial associations between trombiculid mites and bacteria, as well as fungi, were revealed by the *L. deliense* genome project, which identified laterally transferred genes from soil microorganisms in the mite's nuclear genome [12]. These included genes encoding carotenoid synthases-cyclases originating from zygomycete fungi, which were

first reported from genome of the spider mite *Tetranychus urticae* (Tetranychidae) and are responsible for its bright coloration [60]. Moreover, a larger set of laterally transferred genes in the *L. deliense* genome were predicted to confer the ability to synthesize secondary metabolites (**terpenoids**), which is a very rare characteristic among animals. Phylogenetic analyses suggested that the terpene synthases originated from bacterial phyla common in soil (e.g., Actinobacteria, Chloroflexota, Myxococcota, Proteobacteria, Bacteroidetes) and agaricomycete fungi. The potential functions of terpenoids in the biology of trombiculid mites are unknown; however, a role in aversive defence against predators or in sexual reproduction seems most probable when the effects of terpenoids on other arthropods are considered.

The microbiome and genomic studies performed on chiggers to date build a complex picture in which unknown symbiont species (from the Amoebophilaceae, Rickettsiaceae and Neisseriaceae families) dominate in different contexts, while the close association of trombiculids with soil microorganisms (especially in the free-living stages – Figure 1A) can lead to lateral gene transfer and the acquisition of new metabolic functions. Evidently, the unidentified symbionts co-exist in parallel with species of vertically transmitted intracellular bacteria that are widely distributed in arthropods (i.e*., Wolbachia, Cardinium, Rickettsiella*, and *Rickettsia* – Figure 1B). These symbiont genera are well known for inducing reproductive manipulations in their hosts, such as **cytoplasmic incompatibility**, and may be pathogenic to arthropod hosts in some contexts [61,62]. *Orientia* has been reported to impact upon trombiculid development and reproduction, with adverse effects on metamorphosis and fecundity, as well as female-skewed progeny (Figure 1B), observed in some hosts [2,63–66]. However, it should be noted that these studies were performed with no knowledge of other potential reproductive manipulators in laboratory colonies. It is important that changes in the trombiculid microbiome are analysed across the whole life cycle, which has been attempted in only one study to date focused on a single species [49]. Such studies would have to be restricted predominantly to the laboratory colonies at the Armed Forces Research Institute of Medical Sciences in Bangkok due to the substantial difficulties involved in locating adult and nymph samples in the environment, although rearing wild-caught engorged chiggers to obtain the free-living stages is possible [67].

Concluding remarks

Evidently, the number of vertically transmitted bacteria circulating in wild chigger populations provides extensive opportunities for microbe–microbe interactions within trombiculid mites, especially in the reproductive organs (see Outstanding questions). As heritable bacteria can compete during maternal transmission [68,69], studies to determine the effects of the chigger microbiome on vector competence and the population dynamics of *Orientia* in trombiculid populations are urgently needed (see Outstanding questions). Precedents from other arthropod vector systems for potential interference of pathogen transmission by members of the microbiome include *Wolbachia* blocking dissemination of arboviruses in *Aedes aegypti* mosquitoes [70], and the tick symbiont *Rickettsia buchneri* suppressing the growth of rickettsiae that are pathogenic to vertebrates [71]. Undoubtedly, microbiome manipulation or symbiont transinfections in chiggers may never be practicable on a significant scale due to the intractable biology of trombiculids and their extremely limited capacity for self-dispersal. However, a deeper understanding of the chigger microbiome and lateral gene transfers from microorganisms in trombiculid genomes could uncover key vulnerabilities, facilitating the development of novel repellents, pesticides, or even vaccines to control scrub typhus and viral diseases transmitted by these most enigmatic of vectors.

Outstanding questions

What is the importance of chiggers as vectors of pathogens other than *Orientia* spp.?

In hosts other than *Leptotrombidium imphalum*, what is the phenotypic effect of *Orientia* infection on chiggers, and how is it modulated (presumably in reciprocity) by other members of the microbiome?

Beyond colonised *L. imphalum*, how does the trombiculid microbiome change and develop across the life cycle as it shifts from parasitic to free-living mode?

What nonbacterial organisms (parasites, fungi, and viruses) are intimately associated with chiggers?

There appear to be several vertically transmitted bacteria in chiggers coexisting in the wild – how do they interact?

What are the drivers of chigger microbiome diversity in countries other than Thailand?

What is the anatomical distribution of bacteria in or on chiggers, especially in relation to *Orientia* spp.?

What roles do the widespread arthropod symbionts, for example, *Wolbachia, Cardinium*, and *Rickettsia*, play in trombiculid physiology and reproduction?

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Declaration of interests

The authors declare no competing interests.

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